EFFECTS OF PLANT-GROWTH REGULATORS ON SHOOT DEVELOPMENT AND FIELD SURVIVAL OF FOREST-TREE SEEDLINGS

CARL E. OSTROM[1]

Introduction

The phenomenon of bud inhibition by plant-growth regulators, reported by THIMANN and SKOOG (*16*), has recently been utilized to bring about a delay in shoot growth of various species of horticultural plants. In general, these studies indicate that dormancy of tops of deciduous trees and shrubs can be prolonged for one to several weeks by spraying the dormant twigs with naphthaleneacetic acid and similar growth regulators in the concentration range of about 0.01–0.1% (**6, 8, 10, 12, 13**). HITCHCOCK and ZIMMERMAN (**6**) found that fairly low concentrations (0.02–0.04%) of potassium naphthaleneacetate were effective in delaying the opening of buds of fruit trees if applied during the preceding summer. MARTH (**8**) prevented premature shoot growth of rose bushes in unrefrigerated storage by exposing the dormant plants to vapors of ethyl and methyl esters of naphthaleneacetic acid in concentrations of 0.3–0.4 mg. per cubic foot of air for 1–16 hours, depending upon the temperature.

In preliminary experiments at the Beltsville Forest Laboratory, MAKI (**7**) found that leader development of red, shortleaf, and loblolly pines could be arrested for periods of from several months to a full growing season by soaking the tops of the seedlings for 24 hours in an aqueous solution of 1 gm./l. of indolebutyric acid when the new leaders were several inches long. Leaders of eastern white pine were killed by this treatment.

The use of plant-growth regulators to delay leafing-out of hardwoods and to restrict shoot development of pines appeared to have several potential applications in growing, storing, and planting of forest-tree seedlings. Consequently, a number of applied studies were conducted between October, 1942, and October, 1943, in a survey of the following possible benefits of inhibition of shoot growth: (*a*) Prevention of excess top growth of coniferous seedlings when they must be retained for an extra year in the nursery; (*b*) reduction of the ratio of transpiring to absorbing surface to increase the drought resistance of coniferous seedlings; (*c*) extension of the period of dormancy of shoots in the nursery bed to prolong optimum stage for lifting and planting, and prevention of shoot growth of trees during storage; (*d*) delay of leaf emergence of trees after planting to reduce transpiration while the roots are becoming established.

The studies comprising this survey are divided for convenience according to (*A*) nursery-bed, (*B*) pre-storage, and (*C*) pre-planting treatments.

To obtain additional information from the same tests, the growth regulators were frequently applied in carriers such as wax emulsion, which were intended to

[1] Associate Physiologist, Southern Forest Experiment Station, New Orleans, Louisiana. The work was done at the Northeastern Forest Experiment Station, Philadelphia, Pennsylvania.

serve also as protective foliage coatings. The application of wax emulsions to foliage of trees and shrubs is reported by MILLER, NEILSON, and BANDAMER (9) and by CHADWICK (1) to result in higher survival after transplanting. However, SHIRLEY and MEULI (14) detected no superiority in resistance to artificial drought among pine seedlings sprayed with emulsions of four wax and rubber preparations.

Investigation

A. NURSERY-BED TREATMENTS

1. *Seedlings*

PLAN OF EXPERIMENT.—The object of the nursery-bed treatments was to attempt to find a schedule of application of growth regulators which would partially inhibit shoot growth and thus produce trees of low top-root ratio suitable for planting during the subsequent growing season. The preliminary work by MAKI in 1942 indicated that one application of growth regulator in late spring halted leader development of pine seedlings, but that some species (loblolly and shortleaf pines) renewed leader growth later in the summer. Nine different spraying schedules were tried, therefore, involving both single and repeated sprays at monthly intervals from April through July, 1943, in an attempt to curtail shoot growth partially during the whole season. The trees, furnished by the Maryland Department of Forests and Parks in their College Park Nursery, consisted of one 260-foot bed of 3–0[2] red pine (*Pinus resinosa* Ait.), a similar bed of 2–0 table-mountain pine (*P. pungens* Lamb.), and a 30-foot bed of 1–0 loblolly pine (*P. taeda* L.) (fig. 1).

[2] Age classes of forest planting stock are customarily indicated by two figures, the first of which is the number of years in the seed-bed; the second, the number of years in the transplant bed. In forestry nursery practice, "stock" is a collective term for tree seedlings and transplants.

Treatments applied to red pine and table-mountain pine are shown in table 1. The loblolly pine bed was sufficient for only one trial of each chemical at one concentration. Root pruning was included in the test in order to compare the inhibiting effect of pruning with that produced by growth regulators.

PROCEDURE.—The nursery arrangement provided for two root-pruned and two unpruned bed sections, 4 feet wide and 60 feet long, for each of the two main species. The six combinations of chemical and concentration were randomized among six 10-foot blocks in each 60-foot bed section. The ten spraying schedules shown in table 1 were assigned to ten consecutive segments 1 foot long within each 10-foot block.

Naphthaleneacetic acid and naphthalene acetamide were applied singly in concentrations of 200 and 600 gm./l. A mixture of equal parts of these two substances with equal parts of naphthoxyacetic acid and indolebutyric acid was applied also, in the same total concentrations. Because foresters must deal with many species, mixtures of growth regulators rather than single compounds might eventually be found more dependable for general recommendation.

The growth regulators were all dissolved in water containing 50 gm./l. of lanolin which had been emulsified with 5 gm. of laundry soap. The lanolin concentration was higher than the usual carrier concentration of 5–10 gm./l. The level of lanolin selected was determined by current drought tests in the Shirley-Cass Lake drought machine (15) at the Beltsville Forest Laboratory. These tests showed that resistance of seedlings to artificial drought increased with increasing concentration of lanolin emulsion applied as a protective coating, up to a level of 100 gm./l. or more. However, foliage

injury occurred when the concentration was more than about 70 gm./l. In view of these findings it was desired to test, throughout spring and early summer, the possible injurious effects of one to four applications of the protective coating, at a concentration (50 gm./l.) which was considered safe for foliage application.

The sprays were applied at a rate of about 50 cc. per square foot of bed on April 23–24, May 20, June 19, and July 21, 1943. At the time of initial application, the terminal buds had elongated to an average length of ½–1 inch, except in loblolly pine, where they were only beginning to swell.

FIG. 1.—General view of bed of red pine seedlings sprayed with growth regulators at various times during growing season. Photographed September 9, 1943.

RESULTS.—All the 1-year-old loblolly pines were killed by the sprays in the one

concentration applied (600 mg./l.). The effects of treatment on red pine are shown in table 1.

leaders and needles attained only about two-thirds normal length. The mixture of growth regulators was less effective than

TABLE 1

TOP CONDITION OF RED PINE SEEDLINGS IN NURSERY BED IN SEPTEMBER, 1943, AFTER SPRING AND SUMMER APPLICATION OF GROWTH REGULATORS

CHEMICAL	CONCENTRATION (MG./L.)	ROOT PRUNING	BLOCK NO.	MONTH OF SPRAYING									CONTROL
				April	April May	April May June	April May June July	May	May June	May June July	June	June July	
Mixture*.....	200	Unpruned	1	A†	B	F	F	B	F	F	Y_1	Y_2	A
		"	2	A	F	F	F	B	F	F	Y_2	F	A
		Pruned	3	B	B	F	F	C	F	F	F	F	A
		"	4	B	B	F	F	A	F	F	F	F	A
Mixture.....	600	Unpruned	1	A	D	F	F	D	F	F	Y_1	Y_2	A
		"	2	B	F	F	F	D	E	F	F	F	A
		Pruned	3	A	E	F	F	C	F	F	F	F	A
		"	4	A	E	F	F	E	F	F	F	F	A
Naphthalene acetamide...	200	Unpruned	1	A	B	F	F	B	C	F	Y_1	Y_2	A
		"	2	B	E	F	F	C	E	F	Y_2	F	A
		Pruned	3	B	F	F	F	C	E	F	Y_2	F	A
		"	4	B	E	F	F	B	E	F	Y_1	Y_2	A
Naphthalene acetamide...	600	Unpruned	1	B	F	F	F	D	F	F	Y_1	F	A
		"	2	B	E	F	F	D	D	F	Y_1	F	A
		Pruned	3	B	F	F	F	D	E	F	Y_2	F	A
		"	4	B	F	F	F	D	F	F	Y_1	Y_2	A
Naphthalene-acetic acid...	200	Unpruned	1	A	F	F	F	D	F	F	Y_1	F	A
		"	2	B	F	F	F	D	F	F	Y_2	F	A
		Pruned	3	B	F	F	F	D	F	F	Y_2	F	A
		"	4	B	E	F	F	D	E	F	Y_2	F	A
Naphthalene-acetic acid...	600	Unpruned	1	A	F	F	F	F	F	F	Y_2	F	A
		"	2	B	F	F	F	E	F	F	Y_2	F	A
		Pruned	3	C	F	F	F	F	F	F	Y_2	F	A
		"	4	A	F	F	F	F	F	F	Y_1	F	A

* Equal parts (50 mg./l.) by weight of naphthalene acetamide, naphthaleneacetic acid, naphthoxyacetic acid, indolebutyric acid.

† A: Normal.
B: Half or more of trees slightly stunted; needles and leaders about two-thirds normal size.
C: Most trees with needles less than one-half normal length; leader length nearly normal.
D: Practically all trees with needles less than one-half normal length; usually some trees dead.
E: Same as D, but about one-half of trees dead.
F: Practically all trees dead.
Y_1: Foliage somewhat yellowed; some trees dead.
Y_2: Foliage severely yellowed; many trees dead.

Applications of naphthalene acetamide and naphthaleneacetic acid in April usually caused over-all stunting of the new growth of red pine seedlings. Both

either of the naphthalene compounds; it resulted in stunting in only five of eight replications.

The response to effective treatments

applied May 20, after needles had partly expanded, consisted mainly in stoppage of needle elongation (fig. 2). There was also a slight thickening of leaders, without much retardation of their growth in length. The solutions which inhibited growth without killing the trees were 200 mg./l. naphthaleneacetic acid, 600 mg./l. naphthalene acetamide, or 600 mg./l. of the mixture of indolebutyric acid and naphthalene compounds. Naphthaleneacetic acid at this stage was lethal in concentrations of 600 mg.

Single treatments on June 19 had no observable effect on growth of red pine during the current season, for top development was practically complete by that time. However, all treatments applied then resulted in yellowing of the foliage and in death of some of the trees.

Killing of the uppermost fascicles of old foliage was general among trees sprayed in May and occasional among trees sprayed in April. This injury was most severe on portions not shaded by taller seedlings. The injury apparently was due to the lanolin coating where exposed to the summer sun. Although a coating of lanolin from spraying an emulsion of 50 gm./l. usually was not found injurious to conifers planted in the field in the spring, it evidently is injurious to trees in compact nursery beds when applied in hot weather. Cross-sections of red pine needles under a microscope showed penetration of lanolin through

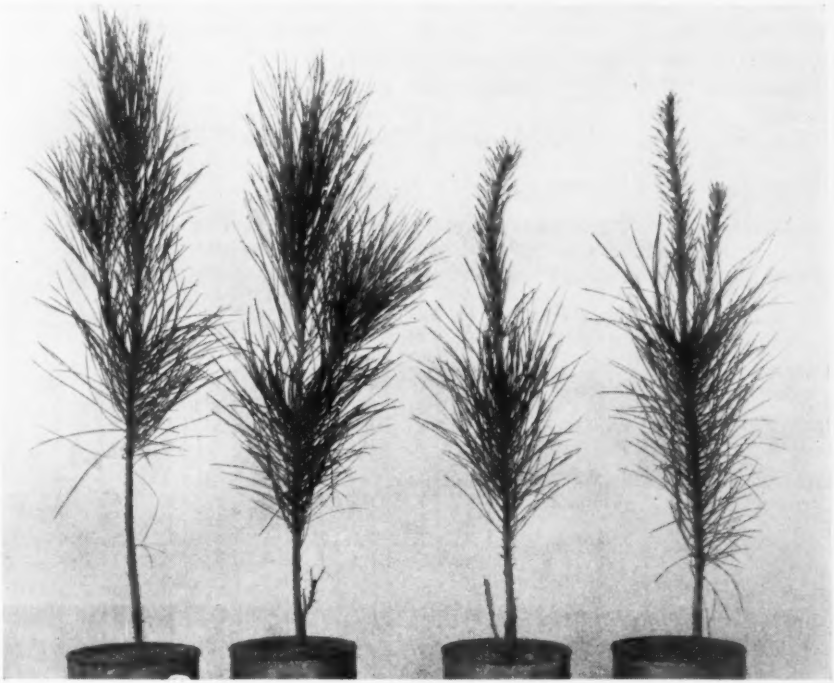


FIG. 2.—Inhibition of needle growth of red pine by two sprayings (April and May) with 600 mg./l. of mixture of growth regulators in 50 gm./l. lanolin. Two untreated controls on left. Photographed July 27, 1943.

the stomata when emulsions containing 50 gm. or more per liter were sprayed on leaves subjected to a temperature of 96°F.

a concurrent study showed severe injury to transplanted trees by several single applications of lanolin only. However, the growth regulators were partly responsible, for the sprays containing the lower concentration of growth regulator were less injurious to the trees (table 1).

TABLE 2

TOP CONDITION OF TABLE-MOUNTAIN PINE SEEDLINGS IN NURSERY BED IN SEPTEMBER, 1943, AFTER SPRING AND SUMMER APPLICATION OF GROWTH REGULATORS

CHEMICAL	CONCENTRATION (MG./L.)	ROOT PRUNING	BLOCK	MONTH OF SPRAYING									CONTROL
				April	April May	April May June	April May June July	May	May June	May June July	June	June July	
Mixture*.....	200	Unpruned	1	B†	B	E	E	B	B, E	F	F	F	A
		"	2	A	A	B, E	F	A	C	B	B	Y2, B	A
		Pruned	3	A	B	E	F	A	E	F	Y1, B	F	A
		"	4	A	A	D	F	A	A	B	Y1	Y1	A
Mixture.....	600	Unpruned	1	A	E	B	F	C	F	F	Y1	F	A
		"	2	A	E	F	F	C	E	F	Y2, B	F	A
		Pruned	3	B	D	F	F	A, D	F	F	F	F	A
		"	4	A	D	F	F	C	F	F	F	F	A
Naphthalene acetamide..	200	Unpruned	1	A	B	F	B, E	A	B, E	F	Y1	Y2	A
		"	2	A	A	F	F	A	B, E	F	Y1, B	Y1, B	A
		Pruned	3	A	B	E	F	A	E	F	Y1, B	F	A
		"	4	A	A	B	F	A	C	C, E	A	Y2	A
Naphthalene acetamide..	600	Unpruned	1	B	E	F	F	C	F	F	Y1	F	A
		"	2	A	E	F	F	C	E	F	Y1	F	A
		Pruned	3	B	E	F	F	A	F	F	Y1, B	F	A
		"	4	A	C	F	F	A, D	F	F	Y1, B	F	A
Naphthalene-acetic acid..	200	Unpruned	1	A	E	F	F	C	F	F	Y1	Y2	A
		"	2	A	E	E	F	C	E	F	Y1, B	F	A
		Pruned	3	A	D	F	F	A, D	F	F	Y2, B	Y2, B	A
		"	4	A	A, D	E	F	A	E	F	Y1, B	F	A
Naphthalene-acetic acid..	600	Unpruned	1	B, E	F	F	F	D	F	F	Y2	F	A
		"	2	B, E	F	F	F	E	F	F	C, E	F	A
		Pruned	3	A	F	F	F	C	E	F	Y1, B	F	A
		"	4	A	E	F	F	D	F	F	Y2, C	F	A

* Equal parts (50 mg./l.) by weight of naphthalene acetamide, naphthaleneacetic acid, naphthoxyacetic acid, indolebutyric acid.

† A: Normal.
B: Half or more of trees slightly stunted; needles and leaders about two-thirds normal size.
C: Most trees with needles less than one-half normal length; leader length nearly normal.
D: Practically all trees with needles less than one-half normal length; usually some trees dead.
E: Same as D, but about one-half of trees dead.
F: Practically all trees dead.
Y1: Foliage somewhat yellowed; some trees dead.
Y2: Foliage severely yellowed; many trees dead.

Two or more applications of the nursery-bed sprays at monthly intervals usually resulted in killing the trees. The injury may have been due to lanolin, for

Table-mountain pine (table 2) re-

sponded essentially as red pine, with one important difference. The leaders of table-mountain pine became irregularly curved as a result of treatment. At the end of the season these leaders were stiffened into somewhat undesirable shapes. Unless this curving can be avoided by some modification of the treatment, the growth regulators may have no value for those pine species which respond in this fashion. Red pine, noted for its strong apical dominance and straight leader, retained its straightness after treatment.

resistance. Red pines were therefore sprayed in April and May, or in May only (1943), with 600 mg./l. of the mixture of growth regulators and lifted at the end of July of the same season for a test in the Shirley-Cass Lake drought machine. This machine consists essentially of a revolving circular table 6 feet in diameter inclosed in an insulated metal drum 4 feet in height. The plants rest on the revolving table and are exposed to a regulated temperature of 96°F., constant light intensity of about 200 foot-candles,

TABLE 3

SIZE AND WEIGHT OF TREATED AND UNTREATED RED PINE SEEDLINGS, 4 MONTHS AFTER MAY APPLICATION OF SPRAY CONTAINING 600 MG./L. NAPHTHALENE ACETAMIDE

Height to base of new growth (inches)	Average length of new leader (inches)		Average dry weight (gm.)		Top-root ratio (dry wt. basis)		Basis (no. trees)	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control
6.1–7.0.....	4.74	5.33	2.94	9.10	3.52	4.10	9	16
7.1–8.0.....	5.85	5.91	4.05	10.33	3.87	5.07	6	11
8.1–9.0.....	5.84	6.46	5.40	16.00	3.93	4.52	12	5
Average..	5.48	5.90	4.13	11.81	3.77	4.56	9	11

Measurements of a sample of red pine seedlings at the end of the season showed that treatment of the tops with 600 mg. of naphthalene acetamide per liter of spray in May had resulted in slightly less leader length, a moderately smaller top-root ratio, and a much lower dry weight per tree (table 3).

The lower top-root ratio of treated seedlings was due chiefly to suppression of new foliage by the growth regulators. Treated seedlings had 60% lower root weight and 66% lower top weight than adjacent untreated trees.

It seemed likely that the smaller foliage area and lower top-root ratio resulting from treatment with growth regulators might cause an increase in drought

and wind velocity of several miles per hour. The table is driven by an electric motor at the rate of 6.6 revolutions per minute. Adjustment of a vent in the side of the drum permits humidity to be maintained close to the 20% level, after the initial high rate of transpiration has subsided.

The trees were graded into four size classes, containing twelve trees per treatment, and potted individually in no. 2 tin cans (fig. 2) containing sand adjusted to 5% moisture content. Examinations of the trees in the drought machine were made at 4-hour intervals (except one interval in early morning), and any trees which had reached the point of permanent wilting, as indicated by previous ex-

perience in reviving wilted trees, were removed from the machine. Results of the test, expressed as average survival time, are given in table 4.

An analysis of variance (4) showed that differences between sizes of plants and between treatments were not statistically significant. This test demonstrated that, despite the smaller foliage area of the seedlings with inhibited shoots, they were no more resistant to artificial drought than were control seedlings at the time the test was made.

TABLE 4

SURVIVAL TIME OF RED PINE SEEDLINGS IN *DROUGHT MACHINE, ACCORDING TO SIZE OF* PLANT AND TIME OF APPLICATION OF SPRAY*

Size	Average survival time (no. of 4-hour periods)			
	Control	Month of treatment		Mean
		April and May	May	
Large	33.2	30.2	29.3	30.9
Medium large	31.1	28.8	30.4	30.1
Medium small	29.3	28.2	30.3	29.3
Small	29.0	22.1	28.0	26.4
Mean (all sizes)	30.7	27.3	29.5	29.2

* Containing 600 mg./l. of mixture of equal parts of naphthalene acetamide, naphthaleneacetic acid, naphthoxyacetic acid, and indolebutyric acid.

In attempts to produce drought-resistant seedlings by inhibition of top growth with growth regulators, it is necessary to consider the possibility that top-pruning also might result in increased drought hardiness. If successful, the latter method would probably be easier for the nurseryman than treatment with chemical sprays. Previous trials of top-pruning of seedlings of ponderosa pine (*Pinus ponderosa* Laws.) by WAHLENBERG (17) before field planting resulted in lower survival among pruned seedlings than among controls. These findings were checked by the drought-machine technique, using second-year loblolly pine seedlings which had been top-pruned in late spring, 1943. Top-pruned and unpruned seedlings from the same nursery bed were divided into comparable size classes (table 5) and placed in the drought machine on August 18, 1943.

TABLE 5

SURVIVAL TIME OF TOP-PRUNED AND UNPRUNED LOBLOLLY PINE SEEDLINGS IN DROUGHT MACHINE

Size	Average survival time (no. 4-hour periods)			
	Degree of pruning			Mean
	Severe	Light	None	
Large	22.9	26.3	35.1	28.1
Medium large	27.7	33.0	39.4	33.4
Medium small	33.2	38.6	41.5	37.8
Small	30.1	42.3	29.8	34.1
Mean (all sizes)	28.5	35.1	36.5	.....

MINIMUM SIGNIFICANT DIFFERENCES BETWEEN MEANS:

Criterion	Significant (P=0.05)	*Highly* significant (P=0.01)
Pruning treatment	3.2	4.2
Size	3.7	4.9
Combination of treatment and size	6.4	8.4

An analysis of variance showed that effects of size class, of degree of pruning, and of the statistical interaction of size and pruning were all significant at the 1% level. Severe top-pruning resulted in a distinct reduction in the resistance of the trees to artificial drought. As in the test of seedlings treated with growth regulators, the smaller foliage area of top-pruned seedlings did not confer

greater drought resistance. The new shoots which developed at or below the point of previous pruning were evidently lacking in drought hardiness, at least at the time of testing.

Some additional top-pruned and unpruned loblolly seedlings were sorted carefully to provide ten trees for each of the size and pruning combinations in table 5. The trees were transplanted in randomized rows in duplicate sand boxes which were watered for a week and then allowed to dry out slowly. After 2 months, survival was 100% for unpruned trees, 72% for lightly pruned trees, and 42% for heavily pruned trees.

These supplementary experiments indicate that top-pruning definitely decreases the drought hardiness of loblolly pine, at least for early fall planting, and support the findings of WAHLENBERG (17) on ponderosa pine.

2. *Transplants*

PLAN OF EXPERIMENT.—To supplement treatments of seedlings with growth regulators in the nursery bed, a smaller test, involving transplants, was made concurrently under different conditions in another nursery at the Beltsville Forest Laboratory. This study included (*a*) single sprays applied to different trees at various times (semi-monthly) throughout most of the growing season, and (*b*) comparison of a commercial-wax emulsion with lanolin emulsion as a protective coating and carrier of growth regulators. The test was designed to determine more closely the effect of time of application on response to growth regulators and protective coatings and to show responses in trees transplanted individually rather than in compact nursery beds.

PROCEDURE.—Six hundred 2–0 loblolly pines and 600 3–0 red pines were transplanted April 9 and 14 to parallel rows, each containing 100 trees spaced 6 inches apart. The following list shows the designation and composition of sprays used:

1. Wax 0.....Commercial-wax emulsion diluted with three parts of water.
2. Wax 200.....Wax emulsion with 200 mg./l. of the mixture of equal parts of naphthaleneacetic acid, naphthalene acetamide, naphthoxyacetic acid, and indolebutyric acid.
3. Wax 600.....Wax emulsion with 600 mg./l. of the mixture of growth regulators.
4. Lanolin 0....Emulsion of 50 gm. of lanolin and 5 gm. of soap per liter.
5. Lanolin 200...Lanolin emulsion with 200 mg./l. of the mixture of growth regulators.
6. Lanolin 600...Lanolin emulsion with 600 mg./l. of the mixture of growth regulators.

The commercial-wax emulsion, according to MILLER *et al.* (9), contained paraffin wax, bentonite, and a fatty acid emulsifier. Each spray was applied to ten trees of each species at seven semimonthly intervals from May 14 to August 17, and to six trees on October 4, 1943. In each row, two unsprayed control trees were left between the groups of trees sprayed at successive intervals. To obtain some information on the lethal limit, an additional lanolin emulsion containing 1 gm./l. naphthaleneacetic acid was applied to twelve extra trees of the second spray on May 29.

RESULTS.—Growth regulators exerted a greater inhibiting action on new shoots when applied in wax emulsion than when applied in lanolin emulsion. Seedlings sprayed with growth regulators after elongation of the shoot but before emergence of the needles eventually had fewer needles emerging than control plants. The several sprays varied in effectiveness, from no inhibition by 200 mg./l.

growth regulators in lanolin emulsion to complete inhibition of needle emergence by 600 mg./l. in wax emulsion.

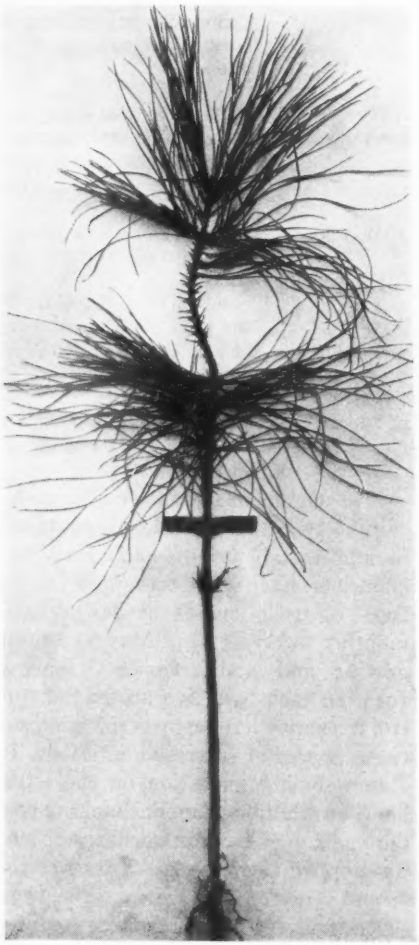


FIG. 3.—Third-year loblolly pine transplant sprayed May 14 with 200 mg./l. of mixture of growth regulators in 1 part commercial-wax emulsion to 3 parts water. Normal growth resumed after initial stunting. Photographed September 23, 1943.

In loblolly pine, inhibition of needle emergence was accompanied by curling of the new shoots, but most of the shoots which were not severely injured had straightened up by the end of the season. Lanolin and wax emulsions without growth regulators had no effect on the emerging leaders.

Growth regulators applied after the beginning of needle emergence checked the expansion of the needles after a few days and resulted in needles of about one-third normal length. A few trees of each species were killed by the sprays containing 600 mg./l. growth regulator. Nearly half of those sprayed with 1 gm./l. naphthaleneacetic acid in lanolin emulsion were killed

The sprays of 200 and 600 mg./l. in lanolin or wax emulsion had no visible effect on red pine when applied after completion of needle elongation, except that all sprays containing lanolin (50 gm. lanolin and 5 gm. soap per liter), when applied on August 4, turned the foliage color of red pine to a yellowish green.

Unlike red pines, loblolly pines normally continue shoot growth through late spring and summer. Treatment of loblolly transplants with growth regulators as late as June, July, or August therefore resulted in modification of subsequent shoot growth. Frequently, when the emergence of needle fascicles was completely prevented by growth regulators, the scale leaf below each fascicle elongated. Shoots of loblolly pine thus modified resembled juvenile leaders which have single needles.

Most of the loblolly seedlings which had been inhibited by growth regulators applied during the early stage of shoot elongation in May resumed normal shoot growth later in the season, as shown by the plant in figure 3. This initial inhibition and subsequent resumption of leader growth would seem at first glance to be advantageous for the survival of planted trees during the first year in the field; top

growth might await the establishment of roots in the new environment, and then proceed normally. Results of field survival studies to test this hypothesis are presented later.

RÉSUMÉ OF RESULTS OF A: NURSERY-BED TREATMENTS

1. Modification of shoot growth of 2- to 3-year-old plants of red, loblolly, and table-mountain pines was brought about by sprays containing 200 mg. naphthaleneacetic acid, 200–600 mg. naphthalene acetamide, or a mixture of several growth regulators per liter of wax or lanolin emulsion.
2. These sprays, when applied just before shoot elongation, usually prevented emergence of most of the new needles but did not prevent the initial surge of leader growth.
3. When applied during the period of needle elongation, the effective sprays arrested needle growth.
4. Inhibited leaders of loblolly and table-mountain pines usually became curved, but red pine leaders remained straight after treatment. Loblolly pines with inhibited leaders resulting from treatment with growth regulators in the spring resumed normal leader growth during the summer.
5. Treated red pines with less than normal foliage area and low top-root ratio were no more resistant than untreated trees to artificial drought during the season in which they were treated. Direct reduction of foliage area of loblolly pine by top-pruning actually decreased resistance of the plants to artificial drought applied several months after pruning.
6. Concentrations of growth regulator which resulted in definite inhibition of the new leaders of pine seedlings usually killed some of the plants. Indications are that one treatment with 600 mg./l. naphthaleneacetic acid is lethal to 1-year-old pine seedlings and that several such sprays will kill older seedlings.

B. PRE-STORAGE TREATMENTS

1. *White ash and loblolly pine*

PLAN OF EXPERIMENT.—Prevention of budding-out of tree seedlings during storage by treatment with growth regulators offers promise of improving survival in the field after late planting. Because no work of this kind had previously been done on species used in forest planting, a rather broad study involving three dates of lifting from the nursery, three types of storage, two dates of planting, and eight growth-regulator treatments was designed in an attempt to define the conditions under which the treatments might be most beneficial in terms of field survival. Advantage was taken of MARTH'S previous work (**8**) in selecting a fairly small number of chemical treatments which could be tried under the various combinations of storage conditions and storage schedules.

The design of the experiment, including all chemical and storage treatments, is shown in table 7. The actual dates of lifting and treating the seedlings were December 1, 1942, and April 15–20, 1943; the dates of planting were May 11–15 and June 16–17, 1943. Half the trees treated and stored in December and half of those treated and stored in April were planted in May, and those remaining were planted in June. All trees treated and stored in May were planted in June. The resulting five periods of storage, applying alike to refrigerated, unrefrigerated, and outdoor conditions, provided fifteen storage treatments, within each of which all the growth-regulator treatments were employed.

PROCEDURE.—The 9000 trees used in this experiment consisted of 1–0 white ash (*Fraxinus americana* L.) and 3–0 loblolly pine. The latter were spindly seedlings of low vigor which later proved extremely susceptible to the shock of transplanting.

Spray solutions were prepared according to MARTH'S method (**8**). Naphthalenemethylacetate and a mixture of naphthalenemethylacetate, naphthalene

acetamide, and naphthoxyacetic acid were employed in total concentrations of 100 and 600 mg./l. in 0.5% commercial-wax emulsion. These solutions were applied with a pressure sprayer to both tops and roots of the seedlings.

Vapors of the same compounds were applied in a large sterilizer of 233-cubic-foot capacity and a special cylindrical chamber of 20-cubic-foot capacity, with water-sealed lid, at the Plant Industry Station at Beltsville Research Center. The growth regulators, in quantities of 0.3 and 0.5 mg. per cubic foot of chamber space, were mixed with alcohol and volatilized from a hot plate, as described by MARTH. Trees were left in the chambers for 16 hours, at 60°–80°F., with roots packed in sphagnum moss. It was considered necessary for purposes of this study to replace the vapor-saturated with fresh moss before storing the seedlings, in order to reduce the danger of contamination among different treatments during storage.

The common and cold-storage facilities were those at the station at Beltsville. The controlled cold-storage room was maintained at 32°F. and at a relative humidity of 85–90%. In the common-storage shed (unrefrigerated) the temperature fluctuated around 40°F. in winter and rose to as high as 70°F. in the summer. The heeling-in bed, in sandy soil under a pine stand, was heavily mulched with pine litter.

In mid-May and mid-June, seedlings of each species were planted in two series of replicate randomized blocks on a plowed area of sandy soil. Each block contained 150 rows, corresponding to the 150 treatment combinations, with fifteen trees per row. Each block was subdivided into five randomized plots corresponding to the five storage schedules.

Loblolly pines planted in May proved to be in very poor vigor. Those left in storage until June had deteriorated so badly that only trees placed in cold storage in December and in April were saved for late planting. These seedlings from cold storage were utilized in a supplementary test of lanolin foliage coatings, for their poor vigor gave promise that any benefits from the foliage coatings would not be obscured by uniformly high survival. The 579 living loblolly pines available for the coating test were separated into two groups equally divided with respect to the original pre-storage treatments, and trees in one group were dipped from tip to root collar in an emulsion of 50 gm. lanolin per liter of water. The seedlings were planted on June 17, with rows of lanolin-coated and uncoated trees completely randomized.

RESULTS.—Of the loblolly pine trees used in the supplementary test of lanolin coatings, the numbers surviving on different dates are shown in figure 4. After 3 weeks (July 5), mortality was 65% among coated trees and 91% among controls. Thereafter, more rapid mortality of the coated trees brought both groups essentially to equality of survival by the end of the season. The summer of 1943 was extremely dry, with merely 23% of normal precipitation throughout July and August (table 6).

Figure 4 shows that seedlings coated with lanolin emulsion had a definite advantage during the early period of transplanting stress; but the continued drought, affecting more and more unprotected new growth, gradually erased the initial advantage. Final judgment as to whether the initial advantage imparted by lanolin coatings would be maintained through a summer of more nearly normal precipitation must be withheld for the present.

Effects of the growth regulators on

white ash were quite evident when seedlings were removed from storage in May for the first planting (table 7).

Untreated trees from common (unrefrigerated) storage bore numerous etiolated new terminal shoots, some as long as $2\frac{1}{2}$ inches, whereas trees sprayed with 600 mg. growth regulator per liter of wax emulsion bore none. Trees treated

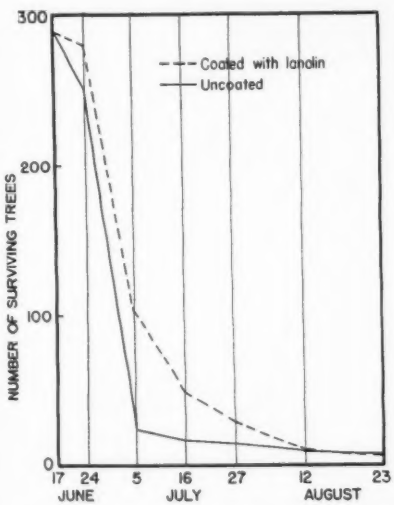


FIG. 4.—Effect of dipping tops of 3-year-old loblolly pine seedlings in emulsion of 50 gm./l. lanolin on seasonal trend of survival in field.

with the low concentration of growth-regulator spray (100 mg./l.) and the high concentration of vapor (0.5 mg./cu. ft.) showed an intermediate stage of shoot inhibition. Of the treated trees exhibiting shoot inhibition, the ones stored in the fall of 1942 usually had a number of short, stout, horizontal roots growing from the lower portion of the stem. Comparably treated trees which had been lifted in April, 1943, and stored for 1 month bore only incipient roots or root swellings along the lower stem. No differences in the small quantity of the normal type of new root growth could be observed between any of the treatment groups removed from common storage in May.

Seedlings removed in May from storage at 32°F. were completely dormant. Those which had been treated in the fall and kept over-winter in the heeling-in bed showed no consistent inhibition of top growth, and seedlings treated with the higher concentrations of growth regulator were in slightly more advanced stage of leafing. Thus the same treatments with growth regulators applied in the fall caused distinctly greater growth responses in trees stored in a shed than in those heeled-in outdoors. Apparently, when trees are stored outdoors over-winter, treatments to delay shoot growth should be applied in the spring rather than in the fall.

TABLE 6

SUMMER PRECIPITATION FOR 1943 AT BELTSVILLE FOREST LABORATORY, COMPARED WITH 40-YEAR AVERAGE AT LAUREL, MARYLAND

MONTH	PRECIPITATION (INCHES)	
	1943	40-year average
May	3.60	3.48
June	2.65	3.99
July	1.20	4.26
Aug.	0.92	4.93
Sept.	4.25	3.56

Seedlings lifted and treated in April showed definite inhibition of shoots for a period of 1–2 weeks in the heeling-in bed, and by May many had developed stout new roots below and just above the root collar.

White ash seedlings removed from cold storage for the second (June) planting were completely dormant. The effects produced in common storage by different

treatments were about the same as those previously recorded, except that the etiolated new shoots had become quite long (figs. 5, 6).

As an example of the condition of the trees at the time of the second planting, the detailed effects of the different treatments on one lot kept in common storage from May 13 to June 14 are given in table 8. The effects of the treatments on field performance, which are discussed in the following section, are also given in table 8 for comparison with other effects of treatment.

Seedlings lifted from the heeling-in bed in June for late planting showed much less inhibition of shoot growth than those held in common storage. The sprays containing 600 mg./l. growth regulator delayed leaf emergence for about 2 weeks, but by June 14 normal top growth was present on most of the trees which had been treated while dormant. Roots which developed on the stems of heeled-in trees as a result of treatment were localized just above the root collar, on that portion of the lower stem that had been covered with soil in the heeling-in bed. The high concentration of spray often caused the initiation of stout new rootlets below the collar also (fig. 7). However, the chemical treatments which caused the most pronounced root initiation often injured the trees, as

TABLE 7

RELATIVE EFFECTIVENESS OF GROWTH-REGULATOR TREATMENTS ON WHITE ASH BASED ON DEGREE OF TOP INHIBITION AND ROOT STIMULATION AT TIME OF REMOVAL FROM STORAGE

Type of storage	Period of storage	Control	Vapor treatments				Wax only	Spray treatments			
			0.3 mg./cu. ft.		0.5 mg./cu. ft.			100 mg./l.		600 mg./l.	
			Nam.*	Mix.†	Nam.	Mix.		Nam.	Mix.	Nam.	Mix.
Cold (32° F.)	Dec.–May						All stock dormant				
	Dec.–June										
	Apr.–May										
	Apr.–June										
	May–June										
Common (40°–70° F.)	Dec.–May	0‡	0	0	0	1	0	2	2	3	3
	Dec.–June	0	0	0	0	1	0	2	2	3	3
	Apr.–May	1	1	2	1	1	1	2	2	2	2
	Apr.–June	0	2	0	3	0	0	2	3	3	3
	May–June	0	2	0	3	2	0	2	3	3	3
Heeled-in outdoors	Dec.–May	1	1	1	1	1	1	1	1	0	0
	Dec.–June	0	0	0	0	0	0	0	0	0	2
	Apr.–May	0	1	0	2	2	0	2	1	3	3
	Apr.–June	0	0	0	0	0	0	0	0	2	1
	May–June	0	3	0	3	2	0	1	1	3	3

* Nam.: naphthalenemethylacetate.

† Mixture of equal parts of naphthalenemethylacetate, naphthalene acetamide, naphthoxyacetic acid.

‡ 0: Active shoot development.
1: Fairly active shoot development.
2: Moderate shoot inhibition, often with root initiation.
3: Severe shoot inhibition, usually with root initiation.

indicated by survival data presented later.

Loblolly pine seedlings failed in general to survive the various storage conditions. Among seedlings heeled-in outdoors over-winter, mortality at the time of removal for the first planting in May was 46.5% for controls and 31.5% for treated seedlings. Although random variation was great, a chi-square test of the ratios of living to dead trees showed that the superiority of the survival of chemically treated trees was of medium significance. However, the growth regulators did not prolong the dormancy of buds of loblolly pine, nor did they stimulate root initiation. Further conclusions based on field survival after planting are given in the following section.

Two weeks after the first (May) planting, there were certain differences in rate of leafing-out of white ash due to the prestorage treatments (table 9).

The pattern of responses in breaking dormancy in the plantation is in most respects similar to the results discussed in the previous section. The mixture of growth regulators applied in the vapor state actually stimulated budding-out of trees lifted in December (when they were relatively resistant to treatment) and kept in cold storage. MARTH (8) also observed stimulation of budding-out by low concentrations of growth regulators in his study of storage of rose bushes. The data in the last line of table 9 summarize the relative effectiveness of the several chemical treatments in prolonging dormancy when applied in the spring before indoor storage. The two spray treatments at a concentration of 600 mg./l. were distinctly injurious; 6 weeks after

TABLE 8

EFFECTS OF GROWTH-REGULATOR TREATMENTS ON WHITE ASH LIFTED MAY 13 AND HELD IN SHED STORAGE UNTIL JUNE 14

Treatment	Length of etiolated shoots			Degree of root initiation‡		Condition in plantation Oct. 5		
	None	0″–6″	6″–12″	Above root collar	Below root collar	Good vigor	Poor vigor	Dead
	(No. trees)					(No. trees)		
Vapor method:								
Control	0	21	9	0	0	5	12	13
Nam.* 0.3 mg./cu. ft.	30	0	0	3	0	13	5	12
Mix.† 0.3 mg./cu. ft.	4	21	5	1	0	13	11	6
Nam. 0.5 mg./cu. ft.	30	0	0	3	3	13	8	9
Mix. 0.5 mg./cu. ft.	28	2	0	2	2	11	9	10
Spray method:								
Wax only	0	18	12	1	0	6	17	7
Nam. 100 mg./l.	28	2	0	2	3	9	11	10
Mix. 100 mg./l.	30	0	0	2	3	15	11	4
Nam. 600 mg./l.	30	0	0	3	3	0	2	28
Mix. 600 mg./l.	30	0	0	3	3	1	1	28

* Nam.: naphthalenemethylacetate.

† Mixture of equal parts of naphthalenemethylacetate, naphthalene acetamide, naphthoxyacetic acid.

‡ 0: None.
1: Incipient.
2: Moderate.
3: Abundant.

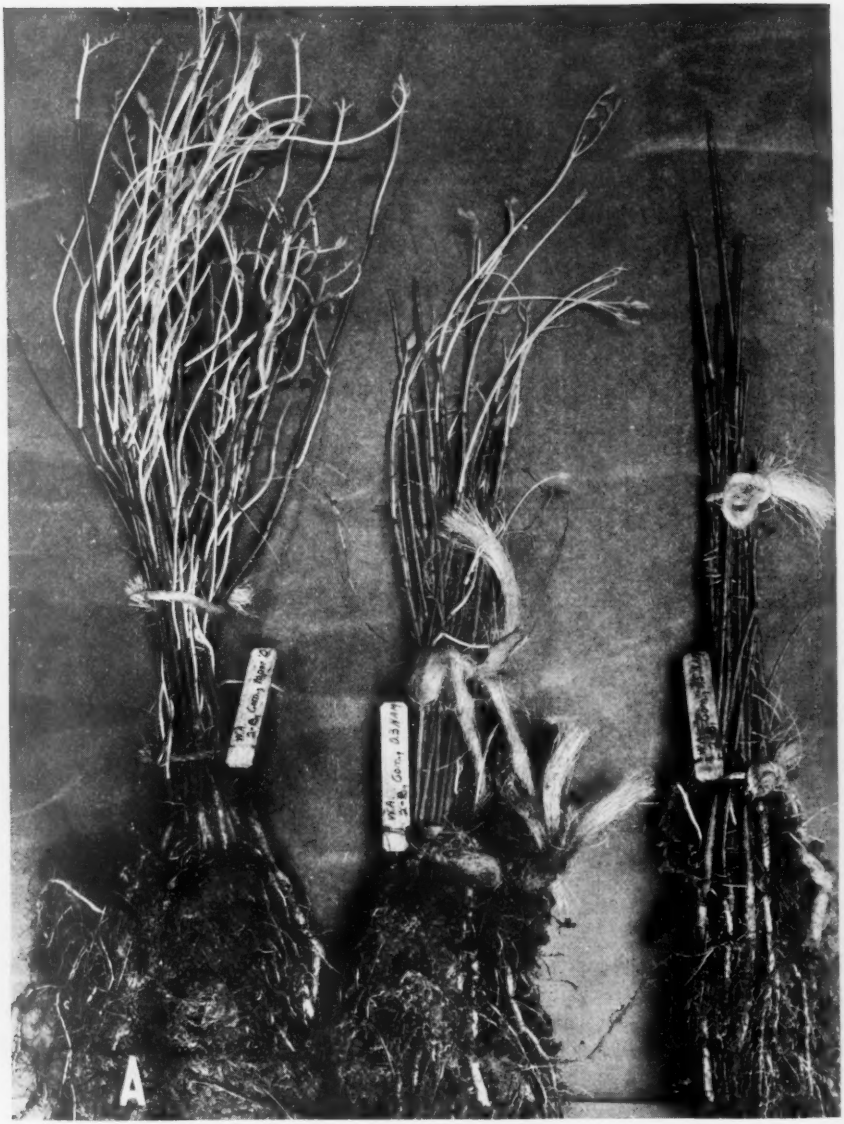


FIG. 5.—Effect of growth regulators on shoot growth and root initiation of white ash seedlings treated April 16 and placed in unrefrigerated storage. *A:* left, untreated; center, exposed 16 hours to vapor of naphthalenemethylacetate (0.3 mg./cu. ft.); right, same but 0.5 mg./cu. ft. *B:* left, untreated; center, exposed 16 hours to vapor of mixture of growth regulators (0.3 mg./cu. ft.); right, same but 0.5 mg./cu. ft. Photographed June 4, 1943.

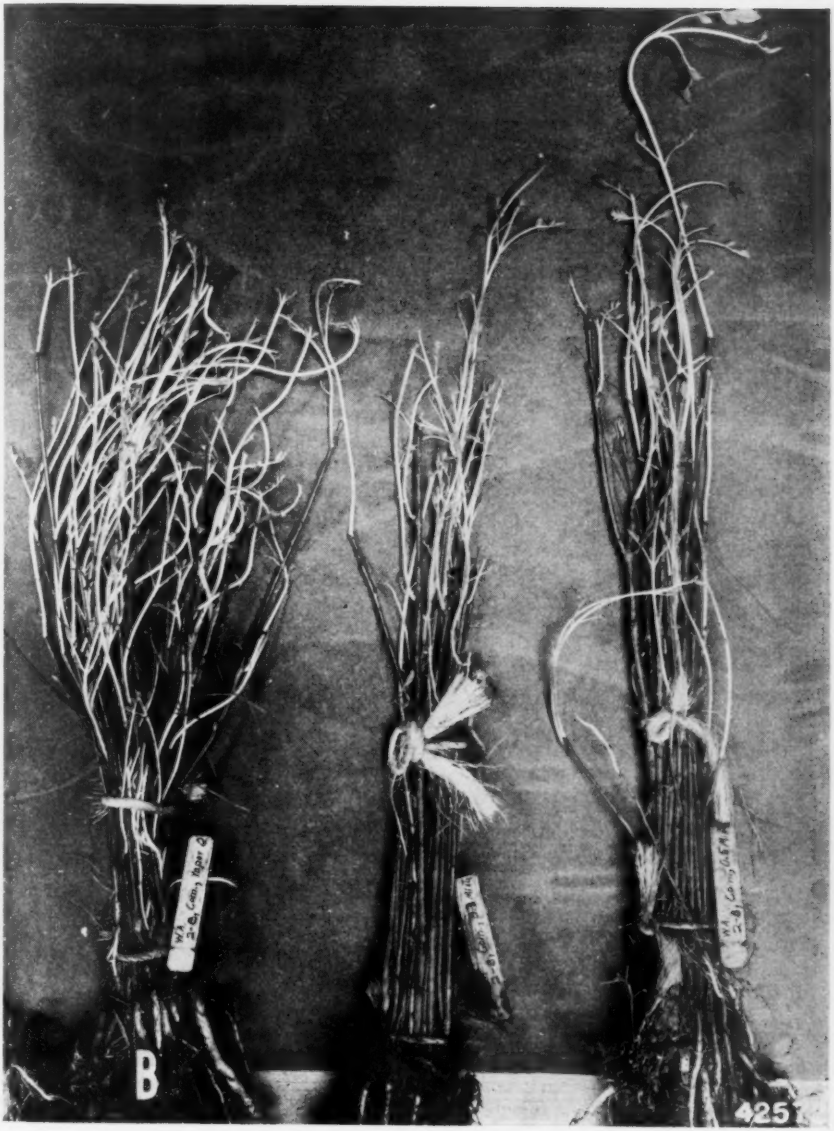


FIG. 5

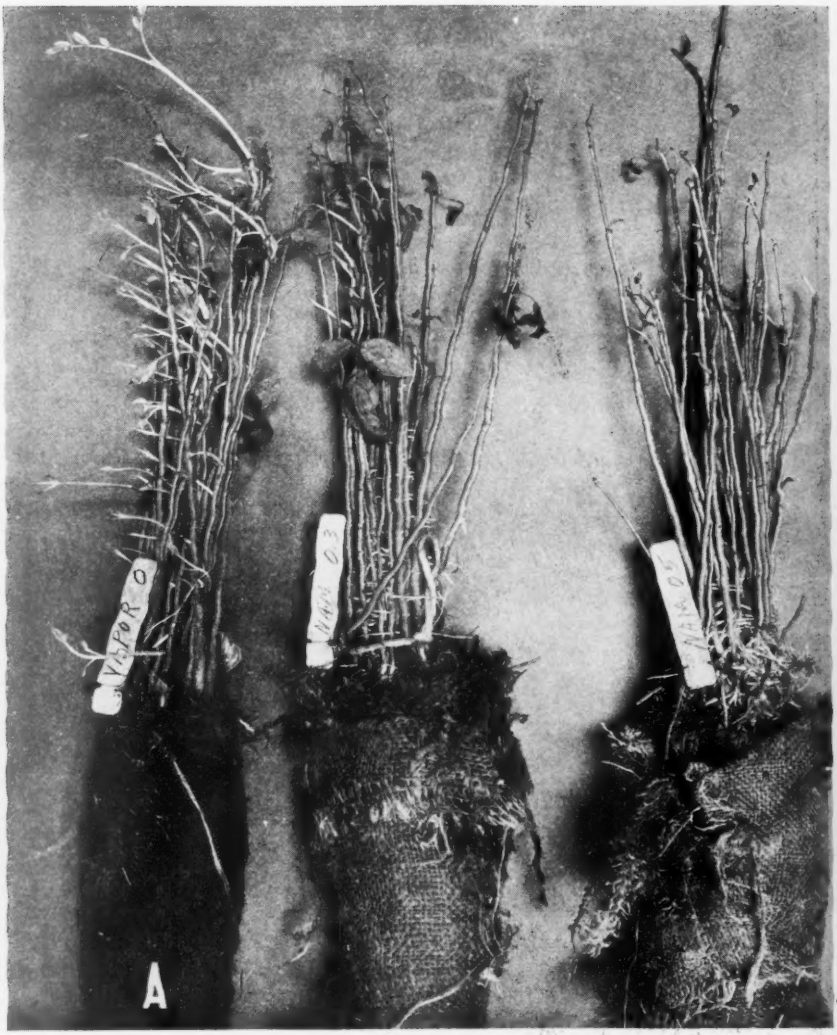


FIG. 6.—Effect of growth regulators on shoot growth and root initiation of white ash seedlings treated May 13 and placed in unrefrigerated storage. *A*: left, untreated; center, exposed 16 hours to vapor of naphthalenemethylacetate (0.3 mg./cu. ft.); right, same but 0.5 mg./cu. ft. *B*: left, sprayed with 0.5% commercial-wax emulsion; center, wax plus 100 mg./l. of mixture of growth regulators; right, wax plus 600 mg./l. of mixture of growth regulators. *Photographed June 4, 1943.*

FIG. 6

planting mortality was 50% for the two concentrated spray treatments and negligible (0–5%) for all the others.

Among trees kept in cold storage, the length of time in storage had more influence on time of leafing than had chemical treatment. Untreated seedlings lifted in April and placed in storage at 32°F. for 1 month practically all leafed out within 2 weeks after planting, but similarly stored seedlings which had been lifted in December budded out much more slowly. The greater readiness of the trees lifted in April was probably due to their exposure to rising temperature in the nursery bed before storage, for control trees lifted in December and kept in warmer shed storage over-winter also leafed out rapidly. At any rate, it appears that hardwood seedlings can be kept dormant longest after removal from cold storage if they are lifted and stored in the fall or possibly in the very early spring.

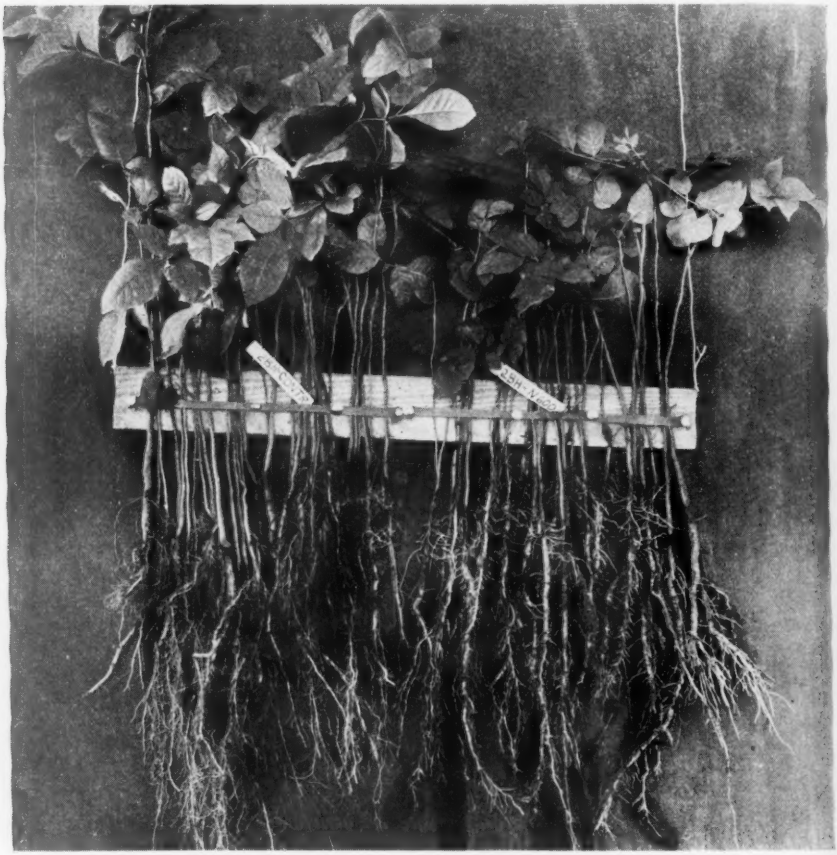


FIG. 7.—White ash seedlings treated April 16 and heeled-in for 2 months. Left, control; right, sprayed with 600 mg./l. naphthalenemethylacetate in 0.5% commercial-wax emulsion, showing stimulation of roots (this concentration injurious to tops). Photographed June 15, 1943.

TABLE 9

NUMBER OF WHITE ASH TREES DORMANT ON MAY 27, 2 WEEKS AFTER FIRST PLANTING. CHEMICAL TREATMENTS AS IN TABLE 8

TYPE OF STORAGE	TIME OF LIFTING AND TREATING	NO. TREES WITH TOPS DORMANT									
		Vapor treatments					Spray treatments				
		Control	Nam. 0.3	Mix. 0.3	Nam. 0.5	Mix. 0.5	Control (wax)	Nam. 100	Mix. 100	Nam. 600	Mix. 600
Cold	Dec.	24*	21	4	15	4	11	9	9	26	30
	April	3	8	5	11	2	1	13	12	30	30
Common	Dec.	1	0	0	1	0	2	1	4	21	17
	April	3	5	1	9	3	2	6	8	29	23
Heeled-in	Dec.	0	0	0	0	0	0	0	1	0	0
	April	2	0	1	2	0	1	1	1	8	6
Total		33	34	11	38	9	17	30	35	114	106
Cold and common	April	6	13	6	20	5	3	19	20	59	53

* Basis: 30 trees per group.

The foregoing results of the early planting were paralleled in many respects by the results of the late planting on June 16 and 17. The condition of the trees 1 week after planting in June is shown in table 10, where the individual chemical treatments have been grouped by three degrees of effectiveness on the basis of all the information so far presented.

The data substantiate the conclusion that dormancy of white ash seedlings was prolonged by cold storage over-winter or by treatment with growth regulators in the spring, particularly if the trees had been stored indoors.

Despite the severe summer drought, mortality was still fairly low when the plantation was re-examined on July 30 and 31. Among trees planted in May, mortality was 40.0% for those treated with the two high concentrations of spray, 3.1% for controls, and 2.2% for the six remaining chemical treatments. Thus, for the early planting, conditions were so favorable for initial establishment that the treatments which prolonged dormancy were of little conse-

TABLE 10

PERCENTAGE OF WHITE ASH TREES WITH TOPS DORMANT IN PLANTATION ON JUNE 21–23 1 WEEK AFTER LATE PLANTING

TIME OF LIFTING AND TREATING	TYPE OF STORAGE	PERCENTAGE DORMANT, BY GROUPS OF TREATMENTS			
		Controls (two groups)	Treated with growth regulators		
			Vapor of mixture* (0.3 mg./cu. ft.)	Effective treatments†	Conc. sprays (600 mg./l.)
Dec.	Cold	63	17	39	82
April		13	10	16	70
May		15	43	69	100
Dec.	Common	0	0	7	27
April		0	0	12	45
May		0	17	50	90
Dec.	Heeled-in	0	0	1	17
April		0	0	1	20
May		2	0	25	85

* Compositions of mixture as in table 8.

† Naphthalenemethylacetate 0.3 mg./cu. ft. (vapor)
Naphthalenemethylacetate 0.5 mg./cu. ft. (vapor)
Naphthalenemethylacetate 100 mg./l. (spray)
Mixture of growth regulators 0.5 mg./cu. ft. (vapor)
Mixture of growth regulators 100 mg./l. (spray)

quence to survival, except that the sprays containing 600 mg./l. growth regulator were definitely harmful.

In the late planting, on the other hand, 20.6% of the control trees died by the end of July, and more severe test of the treatments was obtained. There was an obvious relationship between mortality for different chemical treatments and the effectiveness of the treatments in prolonging dormancy (table 11).

lower than that of trees receiving the weak treatment or no treatment (20.9%).

The superior survival of trees representing the five effective treatments with growth regulators was fairly consistent for all dates of lifting and types of storage (table 11). The relative advantage of the treatments was least among seedlings treated in May and among those stored outdoors. Injury from excessive concentration of growth regulator (600 mg./l.)

TABLE 11

MORTALITY OF WHITE ASH SEEDLINGS JULY 30–31, ACCORDING TO DATES OF LIFTING, TYPES OF STORAGE, AND GROUPS OF CHEMICAL TREATMENTS (JUNE PLANTING)

TIME OF LIFTING AND TREATING	TYPE OF STORAGE	PERCENTAGE MORTALITY, BY GROUPS OF TREATMENTS					
		Controls (two groups)	Vapor of mixture* (0.3 mg./cu. ft.)	Effective treatments†	Conc. sprays (600 mg./l.)	Weighted mean	Basis (no. of trees)
Dec.	All	27.2	26.7	16.7	49.4	26.3	900
April	All	15.0	24.4	9.6	52.8	20.8	900
May	All	19.5	13.3	13.3	57.2	23.3	900
All dates	Cold	13.9	8.9	5.1	36.1	13.4	900
All dates	Common	28.3	34.4	18.9	86.6	35.9	900
All dates	Heeled-in	19.5	21.1	15.6	36.7	21.1	900
Mean		20.6	21.5	13.2	53.1	23.5	2700

* Composition of mixture as in table 8.
† Same as for table 10.

An analysis of variance was performed on the July mortality data, omitting the early planting and the two concentrated sprays, which obviously have separate variances. For this analysis, the numbers of dead trees per group of thirty were transformed to their square roots to reduce the right-skewness of the distributions.

The analysis showed that shed storage resulted in significantly greater mortality than other types. For all types of storage combined, the mortality of trees receiving the five "effective" chemical treatments (13.2%) was significantly

was much greater in common storage than in cold storage or heeling-in beds.

The course of mortality through the summer is shown in table 12 for the late planting only and in figure 8 for all trees in the experiment. The gradual closing of the gap in mortality between the control group and the effective treatments very probably represents an influence of the severe drought of 1943 in masking original treatment differences.

An analysis of variance of mortality to October 6 for late-planted trees, excluding the two injurious spray treatments, showed that differences due to

TABLE 12

SEASONAL TREND OF MORTALITY OF WHITE ASH ACCORDING TO GROUPS OF PRE-STORAGE TREATMENTS; LATE PLANTING ONLY

Date recorded	Percentage mortality, by groups of treatments				
	Control (two groups)	Vapor of mixture* (0.3 mg./cu. ft.)	Effective treatments†	Conc. sprays (600 mg./l.)	Weighted mean
June 21–23	0	0	0.7	16.3	3.6
July 30–31	20.6	21.5	13.2	53.1	23.5
Aug. 23–24	33.5	31.8	28.3	74.1	28.8
Oct. 5–6	35.9	33.7	30.8	75.9	41.1

* Composition of mixture as in table 8.
† Same as for table 10.

growth-regulator treatments were no longer statistically significant. Cold storage was followed by significantly lower mortality (33%) than common storage (51%) or heeling-in (40%).

It was shown previously that control seedlings lifted in December and kept in cold storage over-winter leafed out more slowly than other trees kept in cold storage only in the spring. Comparison of mortality records for these two groups of seedlings revealed no advantage in field survival from the prolonged dormancy which resulted from over-winter storage at 32°F.

Of the white ash seedlings planted in June which survived the first growing season, the proportion in good vigor in October was 47% for controls and 60% for those which had received effective dormancy treatments. On many trees which had been kept until June in common storage, the etiolated storage shoots hardened into crooked shapes after planting. The resulting stem forms of samples of treated and untreated trees are shown in figure 9.

To determine whether growth regulators stimulated root growth after planting, sample rows of trees from one plot planted in June were carefully dug from the plantation in October. The volume of the root and that of the old stem of each tree were measured by water displacement. The volume of the old stem

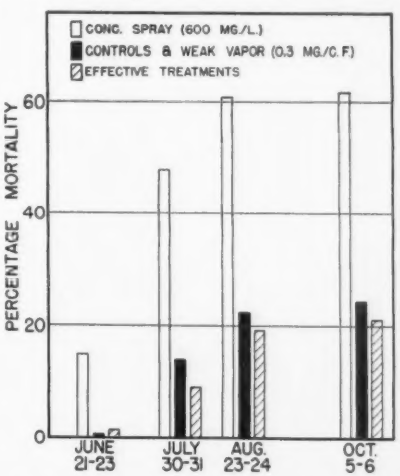


FIG. 8.—Seasonal trend of mortality of white ash seedlings according to groups of pre-storage treatments with growth regulators (May and June plantings combined). "Effective treatments" were those listed below table 13.

(from which all current year's growth had been removed) was considered the most suitable measurement to allow for initial differences in size of plant, and to provide top-root ratio. The data are summarized in table 13.

FIG. 9.—White ash seedlings lifted in May, held in common storage 1 month, planted, and then dug up in October, 1943. Left, eleven untreated trees showing crooked form of new shoots originally put forth in storage shed before planting. Right, thirteen trees sprayed with 100 mg./l. of mixture of growth regulators before storage.

The measurements were tested by analysis of covariance (4), using volume of old stem as the independent variable. Variation in top-root ratio among the treatment groups was highly significant, and a further test of the adjusted top-root ratio for seedlings treated with growth regulators against that of the controls showed this difference also to be highly significant. It is concluded that the stimulation of root initiation observed in common storage and in the heeling-in beds continued to be effective in the plantation, and that a stimulus to root growth after planting resulted from most if not all of the effective treatments with growth regulators. A review of literature on the use of growth regulators in transplating, by MITCHELL and RICE (11), indicates that growth of roots of deciduous trees and shrubs was usually stimulated by soaking the roots for various periods in solutions of 10–80 mg./l. indolebutyric acid.

Because mortality in the plantation exceeded 99% among loblolly pine seedlings that had been stored over-winter, these seedlings will be omitted from further consideration. The effect of chemical treatment on the survival of trees lifted and treated in April is shown in table 14.

None of the pre-storage treatments resulted in a consistent increase in survival and most of the chemical treatments were detrimental in this respect. Even seedlings sprayed on tops and roots with wax emulsion containing no growth regulators showed lower survival than controls. Other studies in this series support the conclusions that the commercial-wax spray is detrimental to field survival of conifers when applied to the roots and that this effect is accentuated by the presence of growth regulators.

TABLE 13

STEM AND ROOT VOLUME OF WHITE ASH SEEDLINGS LIFTED IN APRIL, PLACED IN COMMON STORAGE UNTIL JUNE, AND REMOVED FROM PLANTATION IN OCTOBER, 1943. CHEMICAL TREATMENTS AS IN TABLE 8

	Controls (two groups)	Vapor treatments				Spray treatments†	
		0.3 mg./cu. ft.		0.5 mg./cu. ft.		100 mg./l.	
		Nam.	Mix.	Nam.	Mix.	Nam.	Mix.
Av. vol. of old stem (cc.)	2.25	3.40	2.59	2.43	2.80	1.72	2.90
Av. vol. of roots (cc.)	6.32	11.28	8.15	12.84	9.90	7.75	9.67
Av. ratio top/root (vol.)	0.341	0.300	0.286	0.192	0.277	0.213	0.285
Av. ratio after adjustment*	0.333	0.316	0.286	0.191	0.281	0.204	0.291
				0.2615			
Basis (no. of trees)	17	9	13	10	9	8	13

* Adjusted for differences in volume of old stem by analysis of covariance.
† Trees treated with 600 mg./l. spray were nearly all dead.

2. *Shortleaf pine*

PLAN OF EXPERIMENT.—Because of the great number of species used in forest planting, it was desirable to supplement the main experiments with several smaller tests on other species. One of the

smaller tests of pre-storage treatments involved 1420 3-year seedlings of shortleaf pine (*Pinus echinata* Mill.) which were known to have low resistance to storage and transplanting conditions.

PROCEDURE.—The seedlings were lifted April 15, 1943, for treatment with nine solutions or emulsions of growth regulators (table 15). Lanolin and wax emulsions with and without growth regulators were applied with a 5-gallon hand-sprayer to tops, roots, or whole plants.

RESULTS.—When the seedlings were removed from storage, the proportion in low vigor was 20% for control trees, 21% for trees soaked in growth regulators, 44% for trees receiving root spray, and only 1% for trees receiving sprays of lanolin or commercial wax on tops or tops and roots. The growth regulators had no effect on deterioration in storage. Protective coatings retarded visible deterioration of the seedlings when applied to the foliage but were harmful when applied to roots only. Similar trends are shown in the plantation mortality data (table 15), particularly for the initial examination 2 weeks after planting.

TABLE 14

SURVIVAL OF LOBLOLLY PINE SEEDLINGS ACCORDING TO PRE-STORAGE TREATMENT AND TYPE OF STORAGE; TREES LIFTED IN APRIL, PLANTED IN MAY, AND RECORDED JUNE 17
CHEMICAL TREATMENTS AS IN TABLE 8

TYPE OF STORAGE	LIVING TREES PER GROUP OF THIRTY										TOTAL NO. LIVING	LIVING (%)
	Vapor treatments					Spray treatments						
	Control	Nam. 0.3	Mix. 0.3	Nam. 0.5	Mix. 0.5	Control (wax)	Nam. 100	Mix. 100	Nam. 600	Mix. 600		
Cold	11	9	7	11	13	6	2	5	0	2	66	22.0
Common	9	10	10	2	3	3	2	2	0	0	41	13.7
Heeled-in	1	6	4	4	0	2	0	0	0	0	17	5.7
Total	21	25	21	17	16	11	4	7	0	2	124	.....

Other plants were soaked in water solutions of growth regulators. Inasmuch as past studies at this laboratory had shown roots of pines to be more sensitive to growth regulators than tops, the period of soaking was adjusted accordingly; roots were soaked in the water solutions for 1 hour and tops for 6 hours. The solutions containing 1000 mg./l. of growth regulator were not applied to roots because of the likelihood of injury.

The trees were kept at 32° F. in the cold-storage rooms of the Soil Conservation Service at Beltsville for 54 days. They were planted June 15, in randomized rows of ten trees each, in two replicate blocks to determine effects of the treatments on field performance.

The combination of poor stock and intense drought resulted in severe mortality by the end of the season, but there remained a small benefit from protective coatings containing no growth regulators. Soaking in water solutions of growth regulators resulted in lower survival than no treatment.

A supplementary test involved dipping a sample of 150 of the stored trees in an emulsion of 50 gm./l. lanolin at the time of planting. The trees so treated were well distributed among some of the original pre-storage treatment groups.

Two weeks after planting, 63% of the trees dipped before planting were dead or dying, in contrast to 92% dead or dying among comparable lots of trees not dipped.

test the effect of pre-storage treatments with both protective coatings and growth regulators applied to tops only, roots only, and whole plants. The trees were placed in cold storage for $5\frac{1}{2}$ months to

TABLE 15

MORTALITY OF SHORTLEAF PINE SEEDLINGS 2 WEEKS AFTER PLANTING AND AT END OF FIRST GROWING SEASON, ACCORDING TO CHEMICALS AND METHODS USED IN PRE-STORAGE TREATMENTS

Part of plant treated	Water-solution treatments				Spray treatments					Untreated
					Lanolin (50 gm./l.)			Wax (1:3)‡		
	Nac.*		Mix.†		Mix.			Mix.		
	500 mg./l.	1000 mg./l.	500 mg./l.	1000 mg./l.	None	200 mg./l.	600 mg./l.	None	600 mg./l.	
	No. dead 2 weeks after planting									
Tops	8	7	5	6	1	0	1	0	0	11
Roots and tops	8	8	3	8	1	0	2	0	0	6
Roots	10	.....	3	.....	8	10	10	8	11	6
Total	26	(15)§	11	(14)	10	10	13	8	11	23
	No. dead at end of growing season									
Tops	20	20	20	20	13	18	20	17	19	17
Roots and tops	20	20	20	20	19	20	20	14	20	18
Roots	20	.....	20	.....	17	20	20	20	20	20
Total	60	(40)	60	(40)	49	58	60	51	59	55

* Naphthaleneacetic acid.
† Mixture of equal parts of naphthaleneacetic acid, naphthalene acetamide, naphthoxyacetic acid, and indolebutyric acid.
‡ Commercial-wax emulsion diluted with three parts of water.
§ Totals in parentheses are based on groups of forty rather than of sixty trees.

This small test substantiated results of similar tests on other species. It indicated that treatment with inhibiting concentrations of growth regulators before storage is unpromising for pines, but that protective foliage coatings may improve field survival.

3. *Pitch pine and tuliptree*

PLAN OF EXPERIMENT.—This study, like the preceding one, was designed to provide a rather severe test of the possible protection the treatments might afford against losses during storage.

PROCEDURE.—Several thousand seedlings of 3–0 pitch pine (*Pinus rigida* Mill.) and 2–0 tuliptree (*Liriodendron tulipifera* L.) were lifted from the U.S. Forest Service nursery at Parsons, West Virginia, on April 30, 1943. At the time of lifting, the buds of pitch pine were slightly elongated and many of the tulip-

tree buds were just breaking dormancy. Treatments were accomplished by dipping the seedlings for a few seconds in the various emulsions listed in table 16. trees was determined by visual examination (table 16).

RESULTS.—An analysis of variance based on the numbers of dead trees

TABLE 16

MORTALITY OF SEEDLINGS OF PITCH PINE AND TULIPTREE DURING 5½ MONTHS' STORAGE AT 32° F., ACCORDING TO PRE-STORAGE TREATMENTS. BASIS: 100 TREES PER GROUP, IN FOUR BUNDLES OF TWENTY-FIVE TREES EACH

Coating material	Part of plant treated	Conc. of growth regulator (mg./l.)	Percentage mortality during storage							
			Pitch pine				Tuliptree			
			Growth regulator			Mean	Growth regulator			Mean
			None	Nac.*	Mix.†		None	Nac.	Mix.	
Lanolin (50 gm./l.)	Roots	80	12	4	8	8.00	78	80	85	81.00
	Tops	400	7	10	8	8.33	86	73	77	78.67
	Both	160	13	18	17	16.00	82	80	77	79.67
	Mean		10.67	10.67	11.00	10.78	82.00	77.67	79.67	79.78
Controls	None	0	13				90			
Commercial wax (1:3 H_2O)	Roots	80	21	30	13	21.33	83	87	94	88.00
	Tops	400	26	18	20	21.33	84	87	89	86.67
	Both	160	51	42	65	52.67	99	97	97	97.67
	Mean		32.67	30.00	32.67	31.78	88.67	90.33	93.33	90.78
Controls	None	0	9				90			

MINIMUM SIGNIFICANT DIFFERENCES BETWEEN MEANS FOR PITCH PINE:

Criterion	Significant (P=0.05)	Highly significant (P=0.01)
Part of plant	6.2	8.3
Combinations of coating and part of plant	8.8	11.7
Combinations of coating, growth regulator, and part of plant	15.2	20.2

* Naphthaleneacetic acid.

† Mixture of equal parts of naphthaleneacetic acid, naphthalene acetamide, naphthoxyacetic acid, and indolebutyric acid.

After one night of conditioning at 40° F., the trees were transferred to a cold-storage room maintained at 32° F. and 85% relative humidity. On October 20, 1943, the mortality within each group of showed that only the variation between the two coating materials was significant in the case of tuliptree. Lanolin was superior to commercial wax in preventing losses during storage. Judging from the

equality in survival of control trees and those coated with wax, treatment with lanolin was also superior to no treatment.

A separate analysis of variance of mortality in pitch pine showed high significance of variation due to type of coating, part of plant coated, and the interaction of the two. Approximate minimum significant differences for the significant variables are given in table 17. Differences in mortality attributable to the presence or absence of growth regulators in the coatings were not statistically significant.

Coatings of commercial wax increased mortality of pitch pine, particularly when applied to the whole plant. Lanolin had no significant effect on mortality but showed some tendency to raise it when applied with growth regulators to both tops and roots. In general, however, growth regulators had no appreciable effect on the condition of the stock after $5\frac{1}{2}$ months in cold storage.

Treatment with lanolin emulsion resulted in slightly more mold growth on the roots in comparison with the control trees. The commercial-wax emulsion resulted in moderate to severe molding when applied to roots, probably because of the supply of nitrogen provided by ammonia, which is detectable in the odor of the wax emulsion.

In a test of field survival of pitch pine seedlings treated as described, stored at 32° F., and planted June, 1943, thirteen of eighty controls survived until August, compared with thirty-eight of eighty trees sprayed with lanolin emulsion on tops or tops and roots. The remaining treatments (table 16) were of no benefit.

Résumé of results of B: pre-storage treatments

1. Attempts to inhibit the production of etiolated shoots on white ash seedlings kept in unrefrigerated storage by pre-treatment with growth regulators were essentially successful.
2. Treatments which resulted in inhibition of shoot growth were vapors of 0.3 and 0.5 mg. of naphthalenemethylacetate and 0.5 mg. of a mixture of growth regulators per cubic foot of storage space, and sprays containing 100 mg./l. of either the acetate or the mixture. Sprays containing 600 mg./l. of the growth regulators were usually lethal.
3. Inhibition of shoot growth was usually accompanied by initiation of new roots above and below the root collar.
4. Trees treated in the fall and those stored outdoors showed less response to growth regulators than trees treated in the spring and those stored in a shed.
5. Among treated and untreated white ash seedlings removed from storage and planted in May during favorable weather, mortality during the first season was uniformly low and not related to pre-storage treatments with growth regulators.
6. Among similar seedlings kept in storage until June, those which received effective inhibition treatments survived significantly better than untreated seedlings during the first few months after planting. After a severe summer drought, however, the differences in survival were no longer significant by October of the first growing season.
7. The pre-storage treatments resulted in higher initial field survival of white ash seedlings which had been kept in cold storage (32° F.) as well as in unrefrigerated storage, even though all trees remained completely dormant during storage at 32° F.
8. Untreated trees kept in cold storage survived better in the field than trees treated with growth regulators but stored without refrigeration.
9. Treatments with growth regulators described in paragraph 2 above usually resulted in lowered field survival of loblolly pine seedlings.
10. Naphthaleneacetic and a mixture of growth regulators in concentrations of 200–1000 mg./l. applied in various ways to seedlings of shortleaf pine usually resulted in lower field survival than in untreated trees.
11. Application of an emulsion containing 50 gm./l. lanolin to dormant shoots of short-

leaf pine, pitch pine, and tuliptree before storage usually resulted in higher survival of the trees during storage and after planting.

C. Pre-planting treatments

1. *Red, loblolly, and shortleaf pines*

Plan of experiment.—The previous work on use of growth regulators to delay breaking of dormancy and to inhibit top growth suggested that such treatments might have definite value if applied to forest planting stock just before the trees are set out. It is reasonable to suppose that initial survival might be increased if utilization of stored food by the tops and transpiration of water are decreased through inhibition of shoot growth during the early part of the season while the roots are becoming established. Several studies involving preplanting treatment were therefore conducted concurrently to test this hypothesis.

The plan of the first of these studies included soaking and spray applications of growth regulators to red, loblolly, and shortleaf pine, just before fall and spring planting. Some of the same trees were also tested in the drought machine to help explain the results of parallel tests of survival in the field.

Procedure.—Seedlings of red pine (3–0), loblolly pine (1–0), and shortleaf pine (2–0) were removed from the nursery on October 29, 1942, and sorted into three size classes. Within each class there were thirteen groups of six trees, corresponding to the thirteen treatments shown in table 17.

In making up water solutions of growth regulators, the chemicals were first dissolved in a small quantity of 95% ethyl alcohol. Nevertheless, naphthalene acetamide tended to come out of solution, so it was replaced by naphthaleneacetic acid when these tests were repeated in the spring.

Groups of trees designated for top soaking were immersed to the root collar for 18 hours in the water solutions. Sprays were applied after the trees had been potted individually in sand at 6% moisture content. The top-soaked trees were similarly potted, and all were exposed to 96° F. and humidity of 20% in the drought machine. The three sizes of trees were tested separately in three successive runs of the machine.

In these tests, all plants of one species were removed from the machine at the same time, and each tree was classified in one of seven groups, representing various stages, from no wilting to complete wilting. The seedlings were removed to an unheated greenhouse, where they were transplanted to moist sand to determine which would revive.

Seedlings intended for similar drought-machine tests in the spring were lifted on April 10, 1943, sorted, and treated as before. However, the quantity of lanolin in the sprays was raised to 30 gm./l., for the fall tests had shown that lanolin alone, even in the previous carrier concentration of 5 gm./l., caused a slight increase in drought resistance. After treatment with the solutions and sprays, the trees were heeled-in under a dense pine stand on April 17, for later testing in the drought machine. Since the period of the greatest water stress in spring planting probably comes after new growth has begun to develop, it was planned to conduct the machine tests of the heeled-in seedlings at that stage.

Each of the treatments with growth regulators just described was applied to eighty seedlings of each species to test the effect of the treatments on field survival after fall and spring planting. Immediately after treatment, the trees were

planted in randomized rows of twenty trees in replicate blocks according to an appropriate experimental design.

Fall planting was done November 10 and 11, 1942, and spring planting on April 20 following. The concentration of lanolin in the emulsion sprays was raised from 5 gm. to 30 gm./l. in the spring tests, as previously explained.

TABLE 17

NUMBER OF TREES SURVIVING IN GREENHOUSE AFTER TREATMENT WITH GROWTH REGULATORS AND EXPOSURE TO ARTIFICIAL DROUGHT

Application	Species	No. trees surviving							
		Growth regulator						All treatments combined	Controls (not sprayed or soaked)
		Naphthalene acetamide			Mixture*				
		None	500 mg./l.	1000 mg./l.	None	500 mg./l.	1000 mg./l.		
Spray (lanolin emulsion, 5 gm./l.)	Red pine	3†	2	4	3	6	4	22	2
	Loblolly	8	6	3	9	5	7	38	3
	Shortleaf	4	4	2	3	4	3	20	0
Total		15	12	9	15	15	14	80 24.7%	5 9.3%
Soaking (water solution) 18 hours	Red pine	0	2	2	2	2	0	8	.....
	Loblolly	1	1	0	0	0	0	2	.....
	Shortleaf	0	1	0	0	0	1	2	.....
Total		1	4	2	2	2	1	12 3.7%	.....

* Equal parts of naphthalene acetamide, naphthaleneacetic acid, naphthoxyacetic acid, and indolebutyric acid.

† Eighteen trees tested per species and treatment.

RESULTS.—The numbers of trees surviving in the greenhouse 3 months after the fall drought-machine tests are shown in table 17.

Chi-square tests of the ratios of dead and living seedlings showed that survival of the sprayed seedlings (24.7%) exceeded significantly that of controls (9.3%), but that survival of trees which had received the soaking treatments (3.7%) was not significantly different from that of the controls. Any differences in survival between species were purely arbitrary, depending upon the time that the trees of a given species had been removed from the drought machine. Differences between the two chemicals and between the three levels were not significant.

These results were substantiated by a summary of top-condition scores representing prescribed symptoms of drought injury to foliage and stems at the end of the drought-machine tests. An analysis of variance of the scores showed that visible injury by drought was least among trees sprayed with lanolin emul-

sion and greatest among those with tops soaked 18 hours in water or solutions of growth regulators (table 18).

Thus, application of lanolin-emulsion spray of low concentration (5 gm./l.), with or without growth regulators, resulted in greater resistance to artificial drought, whereas soaking of the tops for 18 hours in water or in growth-regulator solution probably decreased drought resistance of the seedlings.

One month after other seedlings treated in the fall of 1942 had been planted in the field, top condition of each tree was recorded by the same scoring system used in drought-machine tests. Red pine trees were in uniformly good condition. An analysis of variance of the top-condition scores for loblolly and shortleaf pine (table 19) showed that variation between treatments was highly significant and attributable to the superiority of shortleaf pine seedlings sprayed with lanolin emulsion over those not treated. Loblolly seedlings were not benefited by the coating.

TABLE 18

SIGNIFICANCE OF DIFFERENCES IN NUMERICAL INDICES OF WILTING OF PINE SEEDLINGS SPRAYED WITH LANOLIN EMULSION OR SOAKED IN WATER SOLUTIONS AND EXPOSED TO ARTIFICIAL DROUGHT. HIGHEST SCORE INDICATES LEAST WILTING. TREATMENTS AS IN TABLE 17

APPLICATION	AV. CONDITION SCORE PER GROUP OF 18 TREES	NO. TREES TESTED	SIGNIFICANCE OF DIFFERENCE* WITH	
			Control	Soaked
Sprayed (lanolin emulsion)	28.3	324	M	H
Soaked (water solution)	20.9	324	M	.....
Control	24.3	54	.....	.....

* H, high (1% level); M, medium (5% level).

TABLE 19

NUMERICAL INDICES OF WILTING AND VIGOR OF PINE SEEDLINGS IN PLANTATION 1 MONTH AFTER TREATMENT WITH FOLIAGE COATING AND GROWTH REGULATORS
HIGHEST SCORE INDICATES LEAST WILTING

APPLICATION	SPECIES	MEAN TOP-CONDITION SCORE PER TREE							
		Growth regulator						Mean for all treatments	Controls (not soaked or sprayed)
		Naphthalene acetamide			Mixture*				
		None	500 mg./l.	1000 mg./l.	None	500 mg./l.	1000 mg./l.		
Spray (lanolin emulsion)	Loblolly	6.32†	6.15	6.20	6.52	6.50	6.80	6.41	6.42
	Shortleaf	5.67	4.92	5.40	5.40	5.27	5.40	5.34‡	4.00
	Mean	6.01	5.54	5.80	5.97	5.89	6.10	5.88‡	5.21
Soaking (water solution)	Loblolly	6.27	6.30	5.00	6.10	6.27	6.42	6.06	6.32
	Shortleaf	4.12	3.70	3.67	3.80	4.47	4.10	3.97	3.77
	Mean	5.19	5.00	4.33	4.95	5.37	5.26	5.01	5.04

* Equal parts of naphthalene acetamide, naphthaleneacetic acid, naphthoxyacetic acid, and indolebutyric acid.
† Basis: 40 trees per group.
‡ Significantly higher than controls (1% level).

By spring, survival of seedlings sprayed with 5 gm./l. lanolin was no longer superior to that of controls or soaked trees. The growth regulators were not beneficial in this respect, and naphthalene acetamide applied by soaking resulted in an increase of 100% mortality.

During the summer, the record drought of 1943 took a very heavy toll of both fall-planted and spring-planted trees. The progress of mortality for red pine planted in the fall combined with all three species planted in the spring is shown in figure 10. Mortality of loblolly

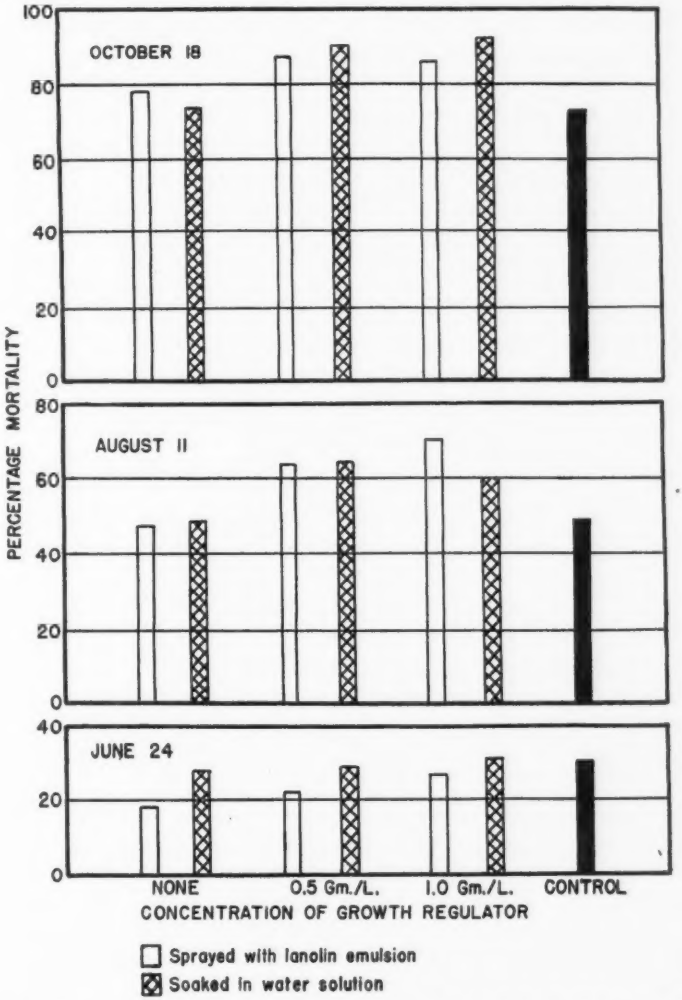


FIG. 10.—Seasonal trend of mortality in seedlings of red, loblolly, and shortleaf pine according to pre-planting treatments with naphthaleneacetic acid or mixture of indolebutyric acid and three naphthalene compounds.

and shortleaf pines planted in the fall of 1942 was so high by the summer of 1943 that they have been omitted from the diagram in order not to obscure treatment differences.

Until the beginning of the summer, trees sprayed with lanolin and planted in the spring had an evident advantage in survival. However, the presence of growth regulator in the coating tended to remove this advantage, probably through direct chemical injury. Later in the season the injuriousness of the growth regulators was clearly reflected in an increased rate of mortality of treated trees. Furthermore, the initial advantage of treatment with lanolin alone was lost, as it was in the test of lanolin coating on foliage of loblolly pine in the main storage study. In both these studies mortality of control trees was 70% or more, and removal of the survival advantage of coated trees was probably due to the effect of the severe midsummer drought on unprotected new growth. In other 1943 plantations, not so seriously affected by the drought, the survival advantage was often maintained through the summer.

The complete pattern of effects of different treatments on survival of the three species at different dates of recording is too complex for detailed consideration, particularly since the growth-regulator treatments usually resulted in lower survival. However, certain facts are obtainable from table 20. Survival of loblolly and shortleaf pine seedlings was very low and was further decreased by nearly all growth-regulator treatments; the decrease was considerably greater for soaked than for sprayed trees. Survival of red pine was relatively unaffected by the treatments, none of which resulted in consistent increase in survival, as recorded in August, 1943.

TABLE 20

SURVIVAL OF RED, SHORTLEAF, AND LOBLOLLY PINES PLANTED IN SPRING AFTER TREATMENT WITH GROWTH REGULATORS, AND RECORDED IN AUGUST, 1943

Application	Species	No. living trees							
		Growth regulator						Mean for all treatments	Controls (not soaked or sprayed)
		Nac.			Mixture*				
		None	500 mg./l.	1000 mg./l.	None	500 mg./l.	1000 mg./l.		
Spray (lanolin emulsion)	Red pine	30†	29	34	27	26	28	29.0	32
	Loblolly	24	1	14	13	21	18	15.2	29
	Shortleaf	13	0	0	11	3	0	4.5	8
	Mean	22.3	10.0	16.0	17.0	16.7	15.3	16.2	23.0
Soaking (water solution)	Red pine	29	35	16	33	35	36	30.7	31
	Loblolly	27	0	0	36	6	1	11.7	27
	Shortleaf	4	2	0	7	0	0	2.2	2
	Mean	20.0	12.3	5.3	25.3	13.7	12.3	14.8	20.0

* Equal parts of naphthalene acetamide, naphthaleneacetic acid, naphthoxyacetic acid, and indolebutyric acid.
† Basis: 40 trees per species and treatment.

Treatment of the dormant seedlings with 500 or 1000 mg./l. of growth regulator before planting caused the same types of shoot inhibition that were described under nursery-bed treatments. The frequency of various degrees of inhibition among the planted trees on June 24 is shown in table 21.

The rather high proportion of control trees which remained dormant or which developed leafless leaders reflected the effects of the transplanting shock and also the rather poor vigor of the loblolly and shortleaf pine seedlings.

All indications are that treatment of pines in the dormant season with 500–1000 mg./l. of growth regulators for the purpose of inhibiting shoot development is likely to reduce field survival rather than to increase it. Apparently, the more severe the top inhibition caused by growth regulators, the lower is the survival of pines. The latter finding is subject to a certain bias, since the trees which showed the greatest inhibition may have been the least vigorous ones originally.

TABLE 21

FIELD PERFORMANCE OF PLANTED RED, LOBLOLLY, AND SHORTLEAF PINES ACCORDING TO LEADER CONDITION IN JUNE, 1943

Treatment	Leader condition on June 24		Condition of same trees, October 23					
	Classification of living trees	Basis (no. living)	Living					Dead (%)
			Dormant (%)	Leader present				
				No leaves		Reduced leaf no. (%)	Normal foliage (%)	
				Leader alive (%)	Leader dead (%)			
All growth-regulator treatments combined	Terminal bud dormant	129	0	0	2	0	0	98
	Leader present, no leaves	463	0	1	5	4	0	90
	Leader present, reduced leaf no.	92	0	0	0	18	3	79
	Leader present, normal foliage	27	0	0	0	0	(44)*	(56)
All controls combined	Terminal bud dormant	16	0	0	0	(6)	(19)	(75)
	Leader present, no leaves	30	0	0	(3)	(7)	(13)	(77)
	Leader present, reduced leaf no.	85	0	0	0	4	29	67
	Leader present, normal foliage	415	0	0	0	0	36	64

* Parentheses indicate percentage based on small numbers.

Mortality during the growing season was greatest for trees which initially exhibited the most severe inhibition of leader development (right column in table 21). Practically all trees still dormant in June were dead by October. Two-thirds of the chemically treated loblolly pines which had leafless leaders in June and which survived the summer developed new foliage later in the growing season, but only one-fifth of the red pines did so. On red pines, the leaders which were leafless in June usually died back, even though the tree remained alive.

The trees intended for the spring series of tests in the drought machine were heeled-in under a fairly dense pine stand

to permit the treatments with growth regulators to take effect on development of new shoots.

By May 31, 1943, 6 weeks after treatment, the typical inhibiting effects of the chemicals on shoot growth were observed. Both concentrations of growth regulator (500 and 1000 mg./l.) applied to dormant seedlings in the spring had practically prevented leafing-out but not shoot elongation of red pine. In loblolly pine, the higher concentration prevented leafing-out, and the lower concentration reduced the quantity of new foliage per shoot by about two-thirds. Inhibited leaders of loblolly showed the usual tendency to curl, and a few of the leaders were dead. None of the shortleaf pine seedlings had yet leafed out; the trees were therefore left in the heeling-in bed to see what would happen to them as well as to the other pine species.

By June 25, 80% of the chemically treated trees and only 10% of the controls were dead in the heeling-in bed. Naphthaleneacetic acid was somewhat more lethal than the mixture, and the lower concentration was only slightly less toxic than the higher one (table 22). The lanolin spray without growth regulators resulted in slight but inconsistent increase in survival of the heeled-in trees.

TABLE 22

MORTALITY (%)* OF RED, SHORTLEAF, AND LOBLOLLY PINE SEEDLINGS TREATED WITH GROWTH REGULATORS AND LEFT FROM APRIL UNTIL JUNE, 1943, IN SHADED HEELING-IN BED

SUBSTANCE	CONCENTRATION		
	0	500 mg./l.	1000 mg./l.
Naphthaleneacetic acid	9.3	87.0	90.7
Mixture†	12.0	65.7	75.9
Mean	10.6	76.3	83.3

* Basis: 108 trees per group.
† Same as for table 20.

The injurious effects of the growth regulators were distinctly more pronounced in the heeling-in bed than in the plantation (fig. 10). It is likely that the dense shade over the heeling-in bed reduced the vigor of the seedlings and consequently decreased their resistance to inhibiting substances. In view of the fact that treatment with growth regulators to kill undesirable trees, if not to inhibit top growth, should have certain practical applications, it might be observed that dense shade may make the trees much more susceptible to injury by such treatments.

2. *Red spruce and tuliptree*

PLAN OF EXPERIMENT.—The preceding study of three pine species was paralleled by another involving red spruce (*Picea rubens* Sarg.) and tuliptree, in order to broaden the applicability of the results. Lanolin and commercial-wax emulsion were used as carriers for the growth regulators, but always in sufficiently high concentrations to serve as protective coatings as well.

An additional feature of the study was the application of coatings to one series of trees immediately after lifting from the nursery, to prevent desiccation during transportation and handling. A brief investigation has already been reported concerning the use of root coatings in lifting and transplanting trees. MILLER, NEILSON, and BANDAMER (9) dipped roots of 2-year-old Norway spruce seedlings in a commercial paraffin-wax emulsion and exposed them to sunshine and wind for periods ranging from 5 to 60 minutes before planting. Survival was several times greater for waxed than for

unwaxed trees exposed in the same manner.

Du Puis (3) found that a solution of 0.1–10% glycerin was effective in protecting the roots of tomato and cabbage plants during storage or shipment. The solution was used in place of water for wetting the packing moss. Chadwick (1) sprayed roots and tops of shrubs with commercial-wax emulsions before transplanting. Top sprays reduced water loss and usually increased survival. Spraying of roots in addition to tops caused a further decrease in water loss but no further increase in survival.

The present study included application of lanolin and commercial-wax coatings to tops, roots, and whole plants, with and without a mixture of equal parts of naphthaleneacetic acid, naphthalene acetamide, naphthoxyacetic acid, and indolebutyric acid. Half the trees were treated just after lifting and the remaining half just before planting or heeling-in. Within each of these two groups, half the seedlings were planted after shipment from the nursery and half were heeled-in 2½ weeks before planting. The four resulting schedules for treatment and planting constituted replications of the experiment under slightly varying conditions. The twelve treatments with chemicals and carriers, applied alike within each replication, are shown in table 23.

Procedure.—Trees for this experiment, provided by the Forest Service Nursery at Parsons, West Virginia, consisted of 2-year-old seedlings of red spruce and tuliptree. The seedlings were lifted April 30, 1943, sorted, and half of them were immediately treated in the packing house by dipping in the emulsions (table 23) for several seconds. Because of rainy weather, the roots had no chance to dry out between the time of lifting and the time of treating.

Similar pre-planting treatments were applied to the remaining half of the seedlings on May 5 and 6, after they had been packed and transported 200 miles to Beltsville. The two species were planted on separate (though adjacent) areas because a statistical comparison of the performance of a conifer and a hardwood is usually precluded by the unlike ranges of the data on survival and growth. Half the trees were planted May 6 and 7, and the remainder were heeled-in until May 24 before being planted. The plantation provided for randomization and replication of groups of trees which had been treated alike.

Results.—Ten days after the early planting, a record of the top condition of all planted trees revealed that growth regulators applied to tops only or to roots and tops prolonged the dormancy of a portion of the seedlings of tuliptree (table 23).

The effects of the three different treatments with growth regulators on rate of leafing-out of additional trees which had been kept in the laboratory basement are shown in figure 11.

In red spruce, rate of leafing-out was not affected by the treatments with growth regulators, but any coating applied to the roots was soon reflected in browning or death of some of the trees after planting (table 24).

The root coatings evidently impeded the absorption of water, and unless the tops were also coated, the trees were subject to internal drought stress. Foliage coatings tended to result in higher initial survival of spruce in the plantation but not in the shaded heeling-in bed, where transpiration was much lower and where very few uncoated trees died.

When the heeled-in trees were removed for planting on May 24, those of red spruce showed no visible response to the growth regulators. As in the plantation, dormancy of tops of tuliptree seedlings was more prolonged among those treated, and a slight increase in growth of new roots was observed in groups with roots previously dipped in the chemicals.

The results of mortality counts of the planted red spruce trees in June are given in figure 12. Although random variation was rather high, as shown by the differences among control groups, mortality to June was distinctly lower in groups treated on tops only with the plain foliage coatings.

Mortality of red spruce seedlings re-

TABLE 23

DORMANCY OF TULIPTREE SEEDLINGS TREATED WITH PROTECTIVE COATINGS AND GROWTH REGULATORS, PLANTED MAY 6–7, 1943, AND OBSERVED 10 DAYS LATER

TREATMENT	PERCENTAGE DORMANT			
	Part of plant treated and conc. of growth regulator			Untreated
	Roots (80 mg./l.)	Roots and tops (200 mg./l.)	Tops (400 mg./l.)	
Lanolin emulsion only (50 gm./l.)	18†	10	3	8
Commercial-wax emulsion only (1:3 H_2O)	12	15	10	6
Lanolin plus growth regulator*	13	28	33	9
Commercial wax plus growth regulator*	10	22	34	9

* Mixture of equal parts of naphthaleneacetic acid, naphthoxyacetic acid, naphthalene acetamide, and indolebutyric acid.

† Basis: 100 trees per group.

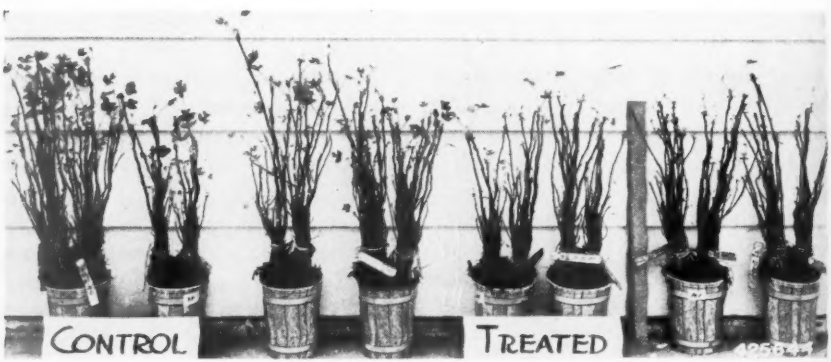


FIG. 11.—Differences in rate of leafing-out of tuliptree seedlings after dipping in emulsion containing mixture of indolebutyric acid and three naphthalene compounds. Reading from left: first pair, untreated; second pair, 80 mg./l. on roots; third pair, 200 mg./l. on roots and tops; fourth pair, 400 mg./l. on tops. Photographed May 15, 1943.

corded on August 24, and the results of an analysis of variance of the data, are given in table 25. The drought of 1943 had already taken a fairly heavy toll ing from treatment of only the tops with plain lanolin or wax emulsion, and significantly higher mortality (49.5%) resulting from treatment of whole plants

TABLE 24

MORTALITY AND BROWNING OF RED SPRUCE SEEDLINGS TREATED WITH PROTECTIVE COATINGS AND GROWTH REGULATORS, PLANTED MAY 6–7, 1943, AND OBSERVED 10 DAYS LATER. CHEMICAL TREATMENTS AS IN TABLE 23

TREATMENT	PERCENTAGE DEAD OR BROWNED			
	Part of plant treated and conc. of *growth regulator*			Untreated
	Roots (80 mg./l.)	Roots and tops (200 mg./l.)	Tops (400 mg./l.)	
Lanolin emulsion only (50 gm./l.)	19*	1	1	22
Commercial-wax emulsion only (1:3 H_2O)	20	2	0	5
Lanolin plus growth regulator	30	3	0	5
Commercial wax plus growth regulator	37	0	10	6

* Basis: 100 trees per group.

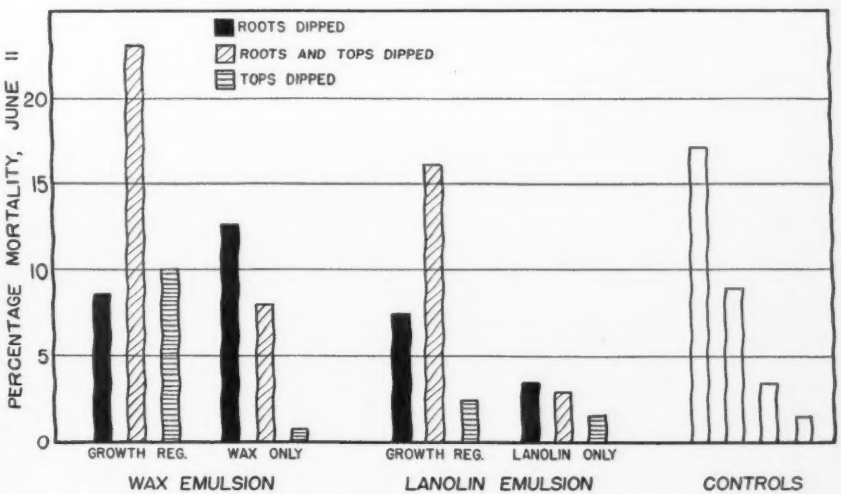


FIG. 12.—Mortality of planted red spruce seedlings in June, 1943, according to pre-planting treatments. *Mixture of indolebutyric acid and three naphthalene compounds applied by dipping roots (80 mg./l.), whole plants (200 mg./l.), or tops only (400 mg./l.).*

among all groups of trees. In comparison with mortality of untreated trees (20.25%), essential differences were significantly lower mortality (9.5%) result- with the protective coatings plus growth regulators. As in other studies with conifers in this series, treatment with commercial-wax emulsion resulted in in-

creased mortality—except when applied without growth regulators to the tops only.

In general, trees treated with coatings and growth regulators just before planting survived better than those treated just after lifting, inasmuch as the growth regulators were more harmful on longer than on shorter contact with the trees.

As in most of the 1943 plantations, the survival of groups of red spruce seedlings treated in different ways tended to approach the same level by fall. Nevertheless, the advantage due to foliage coatings was largely retained through the summer drought. By October 15, average mortality for red spruce seedlings with protective coatings on the tops only was 11.5% compared with 22.2% for untreated trees.

TABLE 25

MORTALITY OF RED SPRUCE SEEDLINGS TO AUGUST 24, 1943, ACCORDING TO PREPLANTING TREATMENTS. DATA FROM TWO MAY PLANTINGS COMBINED. CHEMICAL TREATMENTS AS IN TABLE 23

TREATMENT GROUP	PERCENTAGE MORTALITY				BASIS (TOTAL NO. OF TREES)
	Part of plant treated and conc. of growth regulator			Untreated	
	Roots (80 mg./l.)	Roots and tops (200 mg./l.)	Tops (400 mg./l.)		
Lanolin emulsion only (50 gm./l.)	8.0	20.5	12.5	33.0	800
Commercial-wax emulsion only (1:3 H_2O)	31.5	30.0	6.5	15.0	800
Lanolin plus growth regulator	17.5	41.5	15.5	11.0	800
Commercial wax plus growth regulator	30.0	49.5	34.0	22.0	800
Mean for coatings containing no growth regulator	19.75	25.25	9.50*	24.00	1600
Mean for coatings containing growth regulator	23.75	45.50†	24.75	16.50	1600
Mean for all coatings	21.75	35.375†	17.125	20.25	3200

MINIMUM DIFFERENCES ATTAINING SIGNIFICANCE:

Criterion	Significant (P–0.05)	Highly significant (P–0.01)
Part of plant	7.6	10.1
Combinations of part of plant and presence of growth regulator	10.7	14.2
All controls vs. single combinations of part of plant and presence of growth regulator	9.3	12.3

* Significantly lower than controls (5% level).
† Significantly higher than controls (1% level).

Despite the drought, survival of all groups of tuliptree seedlings was uniformly high (mean 97%), and there was no increase which might have been attributed to the delayed dormancy resulting from treatment of tops with the mixture of growth regulators.

At the end of the growing season, an

analysis of covariance of the length of the old stem and new shoot of 800 tuliptree seedlings showed that the growth regulators and coatings had no significant effect on shoot growth of this species.

Inasmuch as the tuliptree seedlings treated with growth regulators exhibited a slight stimulation of new root growth when they were removed from the heeling-in bed for planting, a sample of trees was dug from the plantation on October 27, 1943, for further study. The sample consisted of four adjacent rows of twenty-four trees each which had been heeled-in just after treatment and then planted in late May. Oven-dry weights of root, of old stem, and of new stem were determined for each tree, and the length of the old stem was measured (table 26).

Weights of both tops and roots were slightly greater among trees treated with the growth regulators, so that the top-root ratios remained about the same as in untreated treees. The total dry weights were analyzed by the covariance method, using length of old stem as an independent variable to remove variation attributable to initial differences in size of trees. This analysis indicated that differences in dry weight between individual treatment groups were not significant, but that the adjusted mean dry weight for all three treated groups combined was significantly higher (5% level) than that of the control group. Thus, treatment with growth regulators resulted in a greater dry weight of the treated tuliptree seedlings, although field survival was equally high among treated and untreated trees.

TABLE 26

TOP LENGTH AND DRY WEIGHT OF TULIPTREE SEEDLINGS AT END OF FIRST SEASON IN PLANTATION, ACCORDING TO PRE-PLANTING TREATMENTS WITH MIXTURE OF GROWTH REGULATORS*

	Part of plant treated and conc. of growth regulator			Untreated
	Roots (80 mg./l.)	Roots and tops (200 mg./l.)	Tops (400 mg./l.)	
Mean length of old stem (inches)	5.82	7.37	5.90	5.86
Mean dry wt. of seedlings in October (gm.)	7.90	9.55	9.05	6.57
Mean top-root ratio, dry wt. basis	0.267	0.272	0.254	0.274
Mean dry wt. of seedlings after adjustment†	8.37	8.29	9.43	7.00
Basis: no. of trees	24	24	24	24

* Same as for table 23.

† Adjusted for initial differences in stem length by analysis of covariance.

RÉSUMÉ OF RESULTS OF C: PRE-PLANTING TREATMENTS

1. Inhibiting concentrations (500 and 1000 mg./l.) of naphthaleneacetic acid and a mixture of growth regulators applied by soaking and spraying just before planting did not affect the field survival of red pine seedlings but resulted in lower survival of loblolly and shortleaf pines.
2. These treatments were usually lethal to similar seedlings kept until June in a shaded heeling-in bed.
3. Dipping the tops of the pine seedlings just before planting in an emulsion of lanolin (30 gm./l.) resulted in increased survival during the spring months but failed to protect the trees against a severe drought which occurred in midsummer. Red spruce seedlings with a similar foliage coating containing

50 gm./l. lanolin survived better than controls throughout the first growing season.

4. A commercial-wax emulsion was detrimental to survival when applied to roots of red spruce seedlings.
5. Dipping tops in lanolin or wax emulsions containing 200 or 400 mg./l. of a mixture of indolebutyric acid and three naphthalene compounds delayed slightly the leafing-out of tuliptree but not of red spruce. The delay in leafing did not result in increased survival of tuliptree after transplanting under favorable conditions, but the treated trees were slightly heavier than control trees at the end of the first year.
6. Red spruce seedlings receiving the same treatments with growth regulators as tuliptree usually showed higher mortality in the plantation than controls.

Discussion

A. Nursery-bed treatment

The prospects for use of growth regulators to prevent excessive top growth of coniferous seedlings in the nursery are not particularly encouraging. In red pine, effective treatments produced trees of distinctly lower dry weight and somewhat lower top-root ratio than controls. The smaller foliage area of treated seedlings did not confer greater drought hardiness during the same summer. It remains to be determined how trees held back in the nursery during one season will perform when planted in the field during a subsequent season.

The primary limitation for use of growth regulators to restrict growth of seedlings in the nursery bed is the danger of killing some of the trees by excessive concentrations of the chemicals, since inhibitory effects are evidently on the borderline of lethality. The curling of leaders of some species of pines (for example, table-mountain pine)after treatment is a limitation in so far as the appearance of the trees is concerned. Another limitation is that growth regulators applied to pines in November and in the spring months did not greatly inhibit elongation of the new leader and did not delay breaking of dormancy. With deciduous trees, however, the prolonged dormancy in treated white ash in the storage studies and in tuliptree in the pre-planting studies could probably be duplicated in the nursery. A slight extension of the spring lifting season would then be possible.

High concentrations of growth regulators afford one means of killing undesirable or excess trees. One or several applications of a wax emulsion or water solution containing 1 gm./l. naphthaleneacetic acid applied during the period of active shoot growth should be lethal to trees in the seedling stage, but these studies give no indication of the concentration required for larger individuals. Studies on herbaceous plants indicate that 2,4-dichlorophenoxyacetic acid is even more effective as a killing agent (**5**).

B. Pre-storage treatment

Perhaps the most promising application of shoot inhibition to forest-planting practice lies in the prevention of premature shoot growth of hardwood seedlings during long storage periods. The resulting increase in field survival of white ash seedlings may have been due in part to stimulation of root growth by the growth regulators, for even trees kept completely dormant at 32° F. survived better in the field if treated with growth regulators before storage. These findings are in agreement with those obtained by Marth (**8**) with rose bushes. The chief limitations shown thus far are the rather wide variation in response of individual trees of the same species and the danger of killing some of the trees. Further experiments on the consistency of the growth response of different lots of seedlings to a given concentration of

growth regulator should precede practical application of the treatments.

Pre-storage treatment of dormant pine seedlings with growth regulators failed to prevent budding-out during storage and appeared to have no advantage.

C. Pre-planting treatment

This survey offers no promise of increased field survival through treatment of pine seedlings with inhibiting concentrations of growth regulators just before planting. Red pine seedlings retain the initial inhibition of leaf growth for the full season, and in this respect they probably characterize the group of northern pines which have only one period of shoot growth per year. Hardwoods, on the other hand, exhibit a delay of only about 2 weeks in shoot growth after treatment, accompanied usually by initiation of additional new roots. One or both of these responses may contribute to increased survival of hardwood seedlings treated with growth regulators before late planting, when premature shoot growth of untreated trees might result in low survival.

Between red pine and similar species which retain the initial inhibition of leaf growth throughout the growing season, and the hardwoods, in which the inhibition is temporary, there is a group of pine species (for example, loblolly pine) which resume normal growth several months after the initial inhibition is induced. The expectation of improved survival of such seedlings, which might result from a temporary reduction in transpiration until the roots became established, was not realized in these studies. In general, treatment of pine seedlings with inhibiting concentrations seemed to reduce the vigor of the seedlings and often killed some of them because of irregularity in sensitivity of individual trees. Although risk of some mortality may be permissible in treating pine seedlings which must be held for an extra year in the nursery bed, this risk could not be afforded in treating trees which are about to be planted.

Dipping the foliage of seedlings of conifers in emulsions of lanolin or commercial wax before planting seems to afford measurable protection from mortality during the spring months, when the trees are becoming adjusted to field conditions. This protection diminishes during the summer months. In the studies reported here the coatings were employed incidentally as carriers of growth regulators; optimum concentrations for protection of foliage were not specifically determined. Application of coatings to the roots was generally of no benefit, and the commercial-wax emulsion increased mortality of coniferous seedlings when applied to the roots. Comar and Barr (2) found that injury to foliage of *Helianthus annuus* sprayed with a wax emulsion was attributable to ammonium linoleate in the spray.

Summary

1. A series of seven experiments, conducted between October, 1942, and October, 1943, involving about 25,000 individual trees and several hundred feet of nursery bed, was undertaken to survey several potential applications to forest-planting practice of the control of top development of seedlings by means of growth regulators. Seedlings of red, loblolly, shortleaf, pitch, and table-mountain pines, red spruce, tuliptree, and white ash were treated in the nursery bed, before storage or before planting. Napthaleneacetic acid, naphthalenemethylacetate, napthalene acetamide, and mixtures of naphthalene

compounds with or without indolebutyric acid were applied in various ways. Methods included dipping, soaking, or spraying with water solutions and carrier emulsions of lanolin or commercial wax, and exposure to vapors of the compounds.

2. Nursery-bed treatments were applied for purposes of prolonging dormancy and restricting the amount of shoot growth. The normal surge of leader growth of pine seedlings was not delayed or substantially lessened by concentrations of 200 or 600 mg./l., sprayed on the expanding buds and old foliage. Needle development, once started, could be completely arrested shortly after treatment and "frozen" for the remainder of the season on red and table-mountain pines. Undesirable curving of the new leaders resulted after treatment of table-mountain pine but not of red pine.

3. At the end of the growing season, red pine seedlings which had been sprayed in May with 600 mg./l. naphthalene acetamide had somewhat lower top-root ratio and much lower average dry weight than untreated seedlings. Inhibiting treatments were close to the lethal concentration, for they occasionally killed some of the trees. More than one application during the period of shoot growth of a spray containing 600 mg./l. naphthaleneacetic acid or similar growth regulators resulted in death of most of the seedlings.

4. Red pine seedlings having only one-half to one-third the normal quantity of new foliage, as a result of inhibition by growth regulators, were no more resistant to artificial drought than untreated seedlings. Top pruning of loblolly pine during early spring resulted in significant decrease in resistance to artificial drought and to transplanting during the same summer and therefore did not appear to be a desirable alternative to growth regulators for checking top growth in nursery beds.

5. Vapors and sprays of naphthalenemethylacetate and a mixture of growth regulators were effective in preventing or restricting the development of etiolated shoots of white ash seedlings held until June in unrefrigerated storage. These treatments also stimulated initiation of new roots, but a high proportion of the new roots appeared on the stem. Inhibition of shoots and stimulation of roots were much less distinct in treated trees heeled-in outdoors. Pre-storage treatments with growth regulators did not affect the survival of white ash seedlings planted in May when controls were still largely dormant, but they resulted in higher initial survival and greater root growth of trees planted in June.

6. For 2-year-old seedlings of white ash, a successful pre-storage treatment was naphthalenemethylacetate applied as vapor (0.3 or 0.5 mg./cu. ft. of space for 18 hours at 70° F.) or as a spray (100 mg./l. in dilute commercial-wax emulsion). Similar pre-storage treatments did not improve survival of loblolly or shortleaf pine seedlings.

7. Growth regulators were also applied just before planting in an attempt to improve initial survival by holding back top growth and transpiration while the roots become established. Pre-planting treatments were applied to tops of seedlings of red, shortleaf, and loblolly pines, and to roots, tops, and whole plants of red spruce and tuliptree. Various treatments with 80-1000 mg./l. naphthaleneacetic acid and other compounds failed to improve the field survival of conifers, and usually lowered it.

8. Seedlings of tuliptree dipped in emulsions containing 200 or 400 mg./l.

of a mixture of growth regulators just before planting in the spring leafed out more slowly than untreated trees. However, survival was so uniformly high for all treatment groups (planted in May) that no advantage resulted from the prolonged dormancy. Tuliptree seedlings treated with growth regulators showed slight stimulation of root growth in the heeling-in bed and excelled controls in total dry weight by the end of the first season in the plantation.

9. Application of emulsions of lanolin (30–50 gm./l.) or commercial wax to foliage of conifers immediately before planting resulted in higher survival during the spring months. After a long and severe summer drought (1943), the survival of coated seedlings exceeded that of controls in some plantations but not in others.

Grateful acknowledgment is due T. E. MAKI of the U.S. Forest Service, who initiated the studies of effects of growth regulators on shoot growth of conifers and supervised the subsequent experiments. Drs. CHARLES HAMNER and PAUL MARTH of the Photoperiod and Hormone Project, Bureau of Plant Industry, Soils, and Agricultural Engineering, aided in designing and applying the chemical treatments and provided the chemicals and storage facilities. Valuable assistance was given by the personnel of Civilian Public Service Camp 34, Bowie, Maryland. HUBERT MARSHALL, ROGER WAY, and Dr. CLARENCE KLINGENSMITH contributed to various technical phases of the survey.

SOUTHERN FOREST EXPERIMENT STATION
NEW ORLEANS, LOUISIANA

LITERATURE CITED

1. CHADWICK, L. C., Data on the use of emulsified paraffins and vegetable oils. Nursery Notes 5:1–5. 1936.
2. COMAR, C. L., and BARR, C. G., Evaluation of foliage injury and water loss in connection with use of wax and oil emulsions. Plant Physiol. 19:90–104. 1944.
3. DU PUIS, R. W., Stimulation of tomato plants by small amounts of glycerine. The Canner 88:16. 1939.
4. FISHER, R. A., Statistical methods for research workers. 7th ed. Oliver and Boyd, London. 1938.
5. HAMNER, C. L., and TUKEY, H. B., Selective herbicidal action of midsummer and fall applications of 2,4-dichlorophenoxyacetic acid. BOT. GAZ. 106:232–245. 1944.
6. HITCHCOCK, A. E., and ZIMMERMAN, P. W., Summer sprays with potassium α-naphthaleneacetate retard opening of buds on fruit trees. Proc. Amer. Soc. Hort. Sci. 42:141–145. 1943.
7. MAKI, T. E., Unpublished report; Northeastern Forest Experiment Station. 1942.
8. MARTH, P. C., Effects of growth-regulating substances on shoot development of roses during common storage. BOT. GAZ. 104:26–49. 1942.
9. MILLER, E. J., NEILSON, J. A., and BANDAMER, S. L., Wax emulsions for spraying nursery stock and other plant materials. Mich. Agr. Exp. Sta. Special Bull. 282. 1941.
10. MITCHELL, J. W., and CULLINAN, F. P., Effects of indoleacetic acid on growth and chemical composition of etiolated bean plants. BOT. GAZ. 99:171–183. 1942.
11. MITCHELL, J. W., and RICE, R. R., Plant-growth regulators. U.S. Dept. Agr. Misc. Publ. 495. 1942.
12. SELL, H. M., REUTHER, W., FISHER, E. G., and LAGASSE, F., Effect of chemical treatments in prolonging dormancy of tung buds. BOT. GAZ. 103:788–793. 1942.
13. SELL, H. M., TAYLOR, H. A., and POTTER, G. F., Effect of chemical treatments in prolonging dormancy of tung buds. II. BOT. GAZ. 106: 215–223. 1944.
14. SHIRLEY, H. L., and MEULI, L. J., Influence of foliage sprays on drought resistance of conifers. Plant Physiol. 13:399–406. 1938.
15. ———, and ———, Influence of moisture supply on the drought resistance of conifers. Jour. Agr. Res. 59:1–21. 1939.
16. THIMANN, K. V., and SKOOG, F., Studies on the growth hormone of plants. III. The inhibiting action of the growth substance on bud development. Proc. Nat. Acad. Sci. Wash. 19:714–716. 1933.
17. WAHLENBERG, W. G., Experiments with classes of stock suitable for planting in the northern Rocky Mountain region. Jour. Agr. Res. 36: 977–1000. 1928.

ALKALOID CONTENT OF ECUADORAN AND OTHER AMERICAN CINCHONA BARKS

WILLIAM E. MARTIN[1] AND J. A. GANDARA[2]

Introduction

Prior to 1942 and the war in the Pacific, more than 90% of the world supply of quinine and related antimalarial alkaloids came from the Dutch East Indies. There on plantations was grown a strain of *Cinchona officinalis* L., sometimes described as Cinchona Ledgeriana Moens. As a result of controlled breeding and selection, clones of much higher alkaloid-producing capacity were developed than have as yet been found in the remaining wild stands now being exploited in South America.

Without access to the East Indies, the United States and its allies found themselves dependent on synthetic antimalarials and on supplies of American bark from the Andean forests of South America. A few plantations had been started in Central and South America, but the majority were too young for harvest and none extensive enough to supply the wartime demands. Exploration parties were therefore sent by the former Board of Economic Warfare, an agency of the United States government,[3] into the Andean countries to search out wild stands of cinchona. Thousands of tons of South American bark have been located and harvested, much of it from areas previously unexplored and unexploited.

The distribution of cinchona in western South America has been found to be very widespread, occurring as a minor constituent of the rain-forests from Bolivia north to Colombia and Venezuela. Where rain-forests occur, cinchona species are found on both east and west slopes of the Andes at elevations of 150 feet to more than 10,000 feet. It will be for the systematic botanist to decide how many distinct species occur in the Andean region and their exact distribution. Much natural hybridization may have taken place and many local varieties or types now exist.

One of the most striking characteristics of cinchona bark chemically is its diverse composition. The layman may think of cinchona as the tree that yields quinine. Actually, quinine is often a very minor constituent or is entirely absent in the bark of many true cinchona species. Three alkaloids of antimalarial value commonly occur in American cinchona barks—cinchonine, cinchonidine, and quinine. In addition, quinidine, an isomer of quinine used in treating auricular fibrillation, is present in small amounts in some barks. Cultivated or improved varieties may yield as much as 13% or 14% of crystallizable alkaloids, with more than 90% in the form of quinine. On the other hand, many wild cinchonas contain mere traces of crystallizable alkaloids and no quinine. The term "total crystallizable alkaloids" is used in this paper as the sum of the per-

[1] Senior Horticulturist, Office of Foreign Agricultural Relations, United States Department of Agriculture. [2] Chemist, Quito Cinchona Analytical Laboratory, U.S. Foreign Economic Administration. This paper is a result of co-operative investigations carried out by the Quito Cinchona Analytical Laboratory of the U.S. Foreign Economic Administration and by the Estación Experimental del Ecuador. The latter is supported by the Republic of Ecuador, the Corporación Ecuadoriana de Fomento, and the U.S. Department of Agriculture, through the U.S. Inter-Departmental Committee on Cultural and Scientific Co-operation.

[3] The Foreign Economic Administration is the successor to the Board of Economic Warfare and Office of Economic Warfare.

centages of anhydrous quinine, quinidine, cinchonine, and cinchonidine.

Cinchonine is very prevalent in native Ecuadoran barks, together with its optical isomer cinchonidine. Some barks contain quinine as well as cinchonidine and cinchonine, while a few contain traces of quinidine. Some barks contain all four alkaloids, others three, while still others have only two. In many cases nothing but cinchonine is present, while in barks of a few true cinchonas none of the four common alkaloids are detectable.

This paper presents data collected in Ecuador by the Foreign Economic Administration and the Office of Foreign Agricultural Relations of the United States Department of Agriculture, together with data from other Latin American countries, which illustrate the comparative variability in composition and alkaloidal content of the species and varieties encountered. The data may be of value in evaluating these species as material for breeding and selection programs in cinchona culture. In addition, data will be presented showing the relative proportions of the several various alkaloids present, which may serve as criteria to support or verify species and variety delimitation.

Investigation

I. Distribution and composition of Ecuadoran cinchona barks

In the course of the operation of the Foreign Economic Administration Cinchona Analytical Laboratory in Quito, more than 1000 bark samples have been analyzed. This total is made up of barks of three principal species—*Cinchona pubescens* Vahl., *C. officinalis* L., and *C. pitayensis* Wedd., together with small numbers of unidentified species as well as species of no commercial value. The approximate compositions of the most common Ecuadoran commercial types within each species are shown in table 1, with analyses of a few other American barks for comparison.

Cinchona pubescens.—This is the most common and widespread species of cinchona in Ecuador. A number of types or varieties may be recognized. The first three of these (table 1) contain only cinchonine, although occasionally samples, perhaps hybrids, are taken which show traces or small amounts of quinine and cinchonidine. The Bofuda type is widespread on the west slope of the Andes from southern Colombia to central Ecuador, at elevations of 5000–8000 feet, but has been little exploited because of its low alkaloid content and lack of quinine. The Rosada type occurs in southern Ecuador on both slopes of the Andes at 4000–8000 feet. Much bark of this type has been harvested for use in totaquina manufacture, in spite of its relatively low alkaloid content and lack of quinine. The Serrana occurs at elevations of 8500–10,000 feet on the west slope in central Ecuador. This regularly contains more than 3% cinchonine and has been extensively harvested in spite of its lack of quinine.

The Roja type of *C. pubescens* is the only commercial type of this species in Ecuador that regularly contains substantial amounts of quinine. It occurs on the western slopes of the Andes, from the Colombian border to southern Ecuador, and in the Colonche Hills—an isolated range between the Guayas Estuary and the Pacific Ocean. It grows at elevations of 2500–4000 feet and has been cultivated for years in small plantations in the Andean foothills of central Ecuador. About 1860, the Englishman Spruce (4) took seeds and seedlings of this type to India, where it was planted under the name of *Cinchona succirubra*.

Extensive plantings were made in Guatemala in the late nineteenth century. One such planting was at Finca El Porvenir, where much natural seeding and regeneration took place. Extensive wild stands developed. These have been harvested and studied during the present war by the Foreign Economic Administration. A comparison of the alkaloidal content of the Guatemalan Succirubra with the Ecuadoran bark of the same variety is shown in table 1. This variety appears to be primarily a cinchonine-producer, as are other types of *C. pubescens*, but with the optical isomer cinchonidine a close second and with quinine usually the minor alkaloid in both Ecuadoran and Guatemalan bark. Samples from Guatemala show slightly higher percentages of each alkaloid than do the limited number of Ecuadoran samples.

TABLE 1

APPROXIMATE COMPOSITION (% DRY WEIGHT) OF ECUADORAN AND OTHER AMERICAN COMMMERCIAL CINCHONA BARKS

SPECIES AND TYPE	SOURCE	NO. SAMPLES	INDIVIDUAL ALKALOIDS PRESENT				TOTAL CRYSTALLIZABLE ALKALOIDS
			Cinchonine	Quinine	Cinchonidine	Quinidine	
C. pubescens Bofuda	Ecuador	36	2.12	.....	.....	.....	2.12
C. pubescens Rosada	Ecuador	165	2.72	.....	.....	.....	2.72
C. pubescens Serrana	Ecuador	60	3.30	.....	.....	.....	3.30
C. pubescens Roja	Ecuador	31	1.96	1.48	1.57	.....	5.01
C. pubescens Succirubra	Guatemala	37	2.47	1.71	2.16	.....	6.34*
C. pitayensis	Ecuador	92	2.63	1.68	0.55	0.19	5.05
C. officinalis Hoja de Lucma	Ecuador	78	1.16	0.41	1.12	.....	2.69
C. officinalis Baños	Ecuador	42	1.36	0.91	1.69	Trace	3.96
C. officinalis Uritusinga	Ecuador	10	0.31	1.42	0.67	.....	2.40
C. officinalis Costrona Fina	Ecuador	32	0.12	1.58	1.06	.....	2.76
C. officinalis Calisaya	Bolivia	52	0.55	3.35	0.83	0.07	4.80†
C. officinalis Ledgeriana	Peru	80	0.69	6.90	1.43	0.29	9.31‡

* Courtesy Foreign Economic Administration Analytic Laboratory, El Porvenir, Guatemala.
† Courtesy U.S. Food and Drug Administration and Foreign Economic Administration.
‡ From Report on Punizas Cinchona Planting (in press).

CINCHONA PITAYENSIS.—This species occurs extensively in southern Colombia and northern Ecuador, at elevations of 8500–10,500 feet on both eastern and western slopes of the Andes. Again, as with *C. pubescens*, this species is primarily a cinchonine-producer but with quinine as the second alkaloid and with cinchonidine present in lesser amounts. Very small amounts of quinidine are usually found. This bark is being extensively harvested, as it has an average of at least 5% total crystallizable alkaloids and is the best source of quinine of any bark at present available in Ecuador.

In leaf characteristics, flower, fruit, and seed appearance, *C. pitayensis* is similar to the Serrana, which is reported as a *C. pubescens* type in central and southern Ecuador in the same elevation zone.

CINCHONA OFFICINALIS.—This species is widespread in southern Ecuador on both eastern and western slopes of the Andes and in central Ecuador on the eastern or Amazon side. A number of distinct types occur at elevations of 4000-

9000 feet. Some, identified as *C. officinalis*, contain practically no alkaloids, while others contain more than 4%. Only the four types extensively harvested will be discussed.

Of the types of *C. officinalis* occurring in southern Ecuador, one (known as Hoja de Lucma) contains on an average equal parts of the optical isomers cinchonine and cinchonidine, with quinine always a minor alkaloid. While this bark seldom contains more than 3% total crystallizable alkaloids, it has been extensively harvested on the eastern slopes of the Andes and to a lesser extent on western slopes and Andean spurs extending toward the Pacific. Recent collections[4] suggest that this may be *C. lucumaefolia* Pavon, by some considered a distinct species, rather than a type of *C. officinalis*. A bark of very similar composition occurs in the Amazon rain-forests of central Ecuador below Baños, where a road penetrates the eastern cordillera and where considerable recent exploitation has taken place. The latter bark contains almost 1% quinine on the average, but usually both cinchonine and cinchonidine exceed the percentage of quinine present.

Among the other *C. officinalis* types of varieties of southern Ecuador, two quinine-producing barks occur. Near Loja a bark known locally as Uritusinga has been harvested since about the year 1640 and marketed as Loja bark, or crown bark, following its use to effect one of the first malarial cures on record in the neighboring town of Malacatos, about 1600 (5). The stands of the Uritusinga variety have been largely cut out, but they originally formed part of the rain-forest at or near the continental divide south of Loja, at 7000–9000 feet. Present samples of this bark are characterized by a rather low alkaloid content, but with quinine as the major alkaloid present and with smaller amounts of cinchonidine and practically no cinchonine (table 1).

In the high Amazon rain-forest east of Cuenca, a bark of similar composition is now being harvested extensively at elevations of 7000–8500 feet. This type, known as Costrona Fina, is quite distinct from the Uritusinga of Loja in leaf, flower, and fruit characteristics, and in its growth. It has almost identical bark composition, however, with quinine as a major alkaloid and small amounts of cinchonidine, while cinchonine is almost entirely absent.

The resemblance of these two Ecuadoran quinine barks to the Calisaya type of *C. officinalis* of Bolivia and the Ledgeriana strain cultivated in Peru is shown in table 1. Costrona Fina and Uritusinga contain little more than half the alkaloid content of the Bolivian samples, but the proportion of the alkaloids present is similar. Also, the cultivated Ledgeriana strain of *C. officinalis* resembles closely the composition of the Calisaya strain, from which it probably arose; but selection and breeding have raised average percentage of quinine and other alkaloids almost to twice that of the parent type, according to the limited number of samples available for comparison.

II. Variation in bark composition

The Ecuadoran commercial samples, the average values of which have been presented in the preceding section, were either group samples taken by field parties to assay cinchona stands encountered in exploration or were aliquot samples taken from lots of bark at time of purchase. Considerable variation—both in total crystallizable aklaloid content and in individual alkaloids—entered the fig-

[4] By W. H. Camp, Field Botanist, Foreign Economic Administration.

ures listed as approximate composition in table 1. In order to evaluate the species and varieties or types for selection or breeding work, or for use in experimental plantings as seedling populations, the amount and type of variation present must be considered.

VARIATION IN COMMERCIAL SAMPLES.—Most Ecuadoran barks have been harvested either as sources of quinine or for use in the preparation of totaquina. This product is a mixture of natural cinchona alkaloids. It contains not less than 7% and not more than 12% anhydrous quinine, and a total of not less than 70% and not more than 80% of the anhydrous crystallizable cinchona alkaloids—quinine, quinidine, cinchonine, and cinchonidine (7). Accordingly, in considering the variability of commercial barks, data both as to total crystallizable alkaloidal content and as to quinine will be presented.

Probably the commonest and most widespread of the Ecuadoran barks are the cinchonine. These rarely contain appreciable quantities of other alkaloids, although occasionally traces of quinine may be found where samples were taken from regions bordering on stands of quinine-producing cinchona types. The commonest cinchonine barks are the Bofuda, Rosada, and Serrana types. Block graphs showing the variability of these common barks in total crystallizable alkaloid content are shown in figure 1. Actually, these values represent only cinchonine.

The Bofuda type is the poorest of the cinchonine barks and shows a variation of from 1% to 4.5% total crystallizable alkaloids. However, 86% of the samples contained 1.5–3.0%. In all, only thirty-six samples of this type of bark were available for study. The Rosada type contains slightly more alkaloids than Bofuda, and 84% of the 164 samples analyzed fell between 2.0% and 3.5%. Both are undoubtedly variant types of *C. pubescens*.

The Serrana type shows (fig. 1) a range from 1% to 5% total crystallizable alkaloids but contains more samples in the higher classes than did either of the other cinchonine barks. Of the sixty samples studied, 77% contained more than 3% total crystallizable alkaloids or cinchonine. This bark has been considered a type of *C. pubescens* and is so listed here because of similarity of composition. In reality, the tree in leaf, fruit, and flower characteristics is much closer to *C. pitayensis*, which occurs in northern Ecuador in the same elevation zone.

On the basis of the commercial samples, it would appear that the Serrana type offers a considerably better possibility for selection of high cinchonine-producers than either Bofuda or Rosada. Alkaloids other than cinchonine are rarely encountered. Cinchonine could be readily and cheaply extracted in pure form by a simple process if there were a demand for it.

The range in alkaloid composition of the four most widely harvested Ecuadoran barks containing quinine is shown in figure 2, which illustrates the variability in alkaloid content. For each bark, the values for quinine are plotted above the corresponding total crystallizable alkaloid values, so that an idea may be gained as to the value of the bark both as source of quinine and as raw material for totaquina manufacture.

The Roja type of *C. pubescens* and the samples of *C. pitayensis* show in figure 2 about the same range of variability. Both populations had some samples of nearly 7% total crystallizable alkaloids. The block graph of the *C. pitayensis* is quite regular and indicates an almost normal distribution of the population of ninety-

two around the central class of 4.5–5.0% total crystallizable alkaloids. The *C. pitayensis* samples contained a little more quinine than those of the Roja type of *C. pubescens*. Only 9% of the thirty-one Roja samples contained more than 2% quinine, while more than 33% of the *C. pitayensis* samples exceeded this value. Neither shows promise as source material for quinine extraction but could well be used in selection work of plant materials suitable for totaquina production.

The two types of *C. officinalis* illustrate a marked difference in alkaloid-forming characteristics. Both the Hoja de Lucma and the Costrona Fina are low alkaloid-producers and show approximately the same range of variability in total crystallizable alkaloid content. However, none of the seventy-eight Hoja

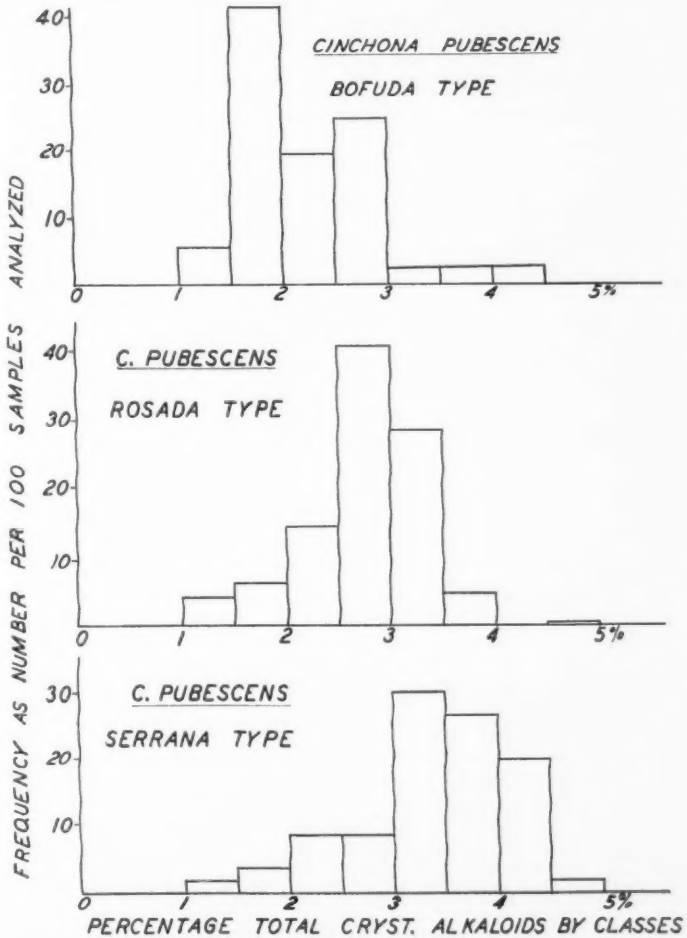


FIG. 1.—Variation in total crystallizable alkaloid content of Ecuadoran cinchonine barks

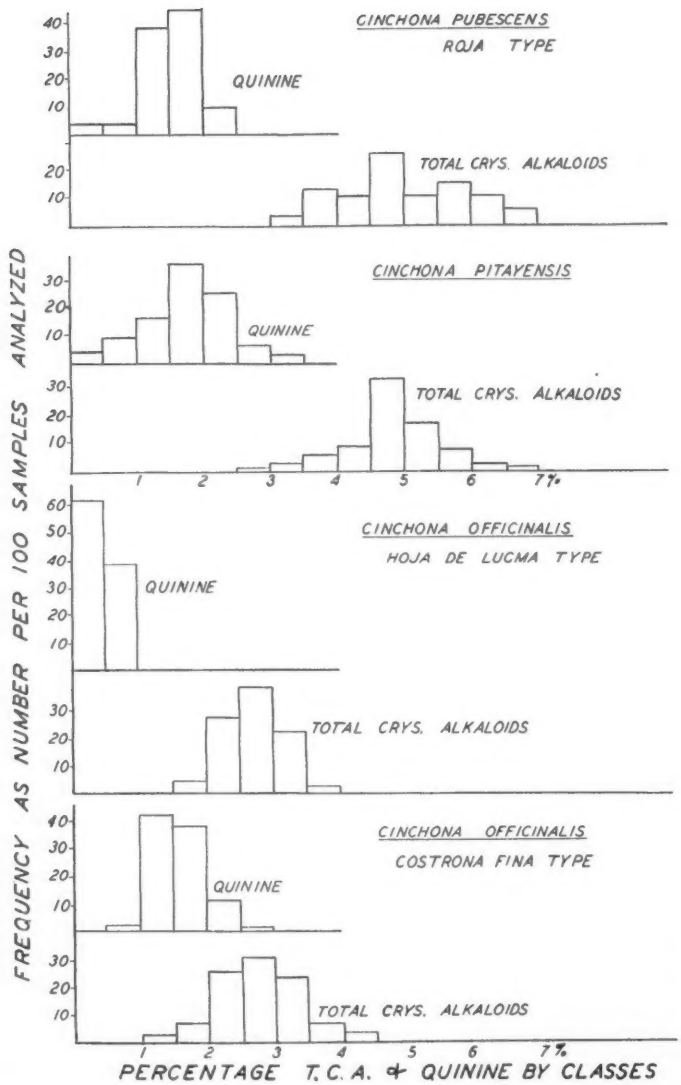


FIG. 2.—Variation in total crystallizable alkaloid and quinine content of Ecuadoran commercial barks

de Lucma samples contained as much as 1% quinine, while nearly all the forty-two Costrona Fina samples exceeded this figure. In this latter type, 82% of the population contained 1–2% quinine, while in 13% of the cases more than 2% quinine was found. As a commercial quinine source such bark is not promising, but it does offer nearly as good a source of quinine as either *C. pitayensis* or the Roja type of *C. pubescens*.

VARIATION IN INDIVIDUAL TREES.—While the assay of commercial group samples may provide information as to the usual composition of a bark, and may indicate the range of variability to be expected, it can hardly serve as an accurate guide in planning selection work to build up clones of high alkaloid-producing capacity. Accordingly, data are presented showing the composition of bark from individual trees of the two most promising types encountered in the preceding section. Also, in figure 3 are data from a group of cultivated trees of the Ledgeriana type of *C. officinalis*.

A group of fifty-seven wild trees of *C. pitayensis* were sampled in a single stand in Ecuador to determine in a preliminary way the possibility or feasibility of clone selection in this species. These were growing in a group of groves mixed with other forest trees along a single ridge at about 9500 feet. As the trees were felled in commercial harvest operations, a bark sample at 1-m. height around the circumference of each tree was taken and softwood cuttings cut from the tree top and placed in nurseries adjacent to the harvest area. Data from the analyses of these bark samples are shown in figure 3. The population sampled was apparently made up of two types of tree, as may be seen from the figure. Nine of the trees contained less than 2% total crystallizable alkaloids, mostly cinchonine, while the remaining trees showed a range from 2% to 8%, much the same as observed in the commercial series of samples of this species (fig. 2). No trees of unusually high quinine content were encountered.

Through the courtesy of the Foreign Economic Administration, data have been made available to secure an assay on the possibilities of clone selection in the Succirubra type of *C. pubescens*. This type of cinchona was widely planted in Guatemala in the late years of the nineteenth century (3). At Finca El Porvenir in northwestern Guatemala it escaped from cultivation, and large wild stands developed in the mountains adjacent to the original plantings. In connection with the propagation program of the Foreign Economic Administration at El Porvenir, a group of 314 wild trees was sampled and analyzed individually in a clone-selection program carried out under the direction of Mr. WILLIAM PENNOCK, Horticulturist, and Mr. WALTER PLOCHARSKI, Chemist. Permission to use their unpublished data for a comparison with other material in this report is gratefully acknowledged.

This population of Succirubra trees varied widely in total crystallizable alkaloid content (fig. 3). Total values for individual trees varied from 1.7% to more than 11.5%. The graph indicates a very nearly normal distribution pattern. Nearly 4% of the population was made up of trees with over 9.5% total crystallizable alkaloids. Quinine analyses were made on bark of 111 of the best trees, and the variation in quinine content (fig. 3) indicates that this type of *C. pubescens* offers little possibility as a commercial quinine source. In no case did the maximum quinine values approach the high total values encountered. It is evident that Succirubra does offer

excellent possibilities for the establishment of clones for the production of high alkaloid bark suitable for totaquina manufacture.

in total crystallizable alkaloids and quinine content is presented in figure 3. A range from 0.3% to 13.0% total crystallizable alkaloids was encountered. Only

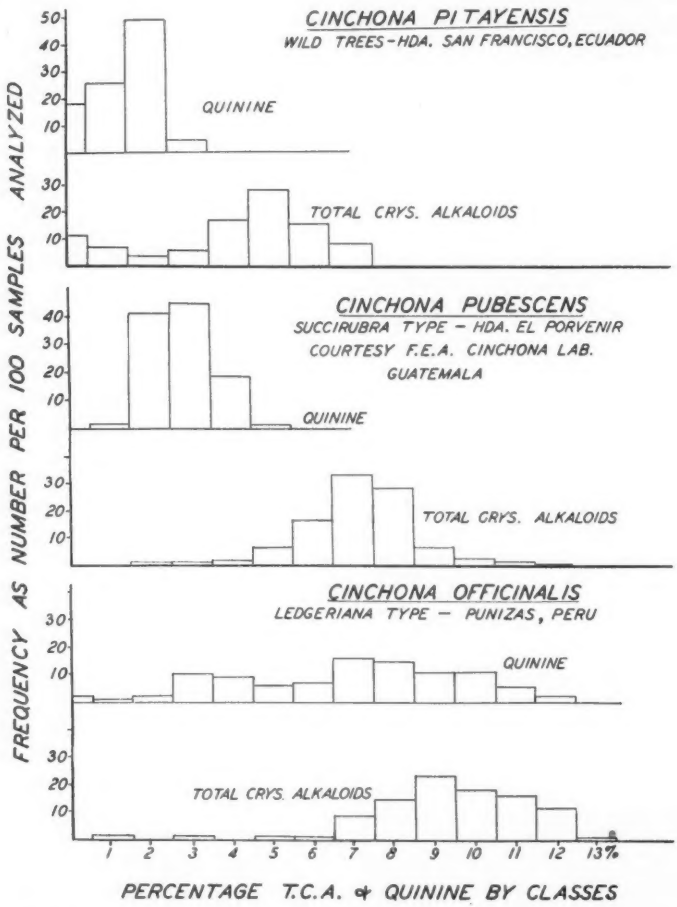


FIG. 3.—Variation in total crystallizable alkaloid and quinine content of bark of individual trees

In 1942, groups of eighty Ledgeriana trees were sampled individually on a small plantation in Peru by MARTIN and CRANDALL (1). The description of the planting and the results of bark analyses form the subject of a separate report (2). For comparison, the reported variability

a small part of this variability could be accounted for as related to age of tree. More than 94% of the population yielded samples of more than 6.5%, while 29% of the trees gave values in excess of 10.5%.

A large proportion of the alkaloids present in the bark of these Ledgeriana

trees was present as quinine. Figure 3 shows that the quinine values in many cases approach the total maximum values. It is reported (2) that in two-thirds of the population, quinine accounted for more than 70% of the crystallizable alkaloids present, while in nearly one-third of the trees more than 90% was found in the form of quinine. Obviously, such a population offers a far better possibility for the selection of quinine-producing clones than does either Succirubra or *C. pitayensis*.

III. Alkaloid ratio diagrams as aid to species and variety delimitation

In the preceding section, data have been presented showing the range of variability in composition of the principal Ecuadoran cinchona barks, together with limited data from other Latin American countries for comparison. In studying these data it was observed in some cases that, where one alkaloid was present in larger amounts than usual, another might be correspondingly low, with the result that the total crystallizable alkaloid value of the sample did not differ materially from that usually typical of the species or type of bark. In other cases within a group of like samples, some of rather low or unusually high total content were observed. In such samples the relative proportions of the constituent alkaloids often remained about the same as that usual for the species or type. This suggested the advisability of considering alkaloid accumulation and formation characteristics apart from the actual percentages and on a relative basis.

Accordingly, the data have been recalculated, expressing the three individual alkaloids—quinine, cinchonine, and cinchonidine—as percentages of the values for total crystallizable alkaloids. In cases where quinidine was present in very small amounts, the alkaloid percentages were calculated from the sum of the three principal alkaloids. The data so obtained have been plotted on triangular co-ordinate charts. In this way it is possible to plot the percentages of quinine, cinchonine, and cinchonidine at the same time. The position of the resultant dot indicates the composition of the sample with respect to the relative proportions of its three constituent alkaloids. Thus, in figure 4, if a dot were to fall at any apex of the triangle it would be composed of but a single alkaloid. On the other hand, if a dot were to fall at the exact center of the triangle, it would represent a sample composed of equal parts of quinine, cinchonine, and cinchonidine. Points falling on the sides of the triangle would be made of only two alkaloids. In figure 4 are plotted the points representing the commercial Ecuadoran samples of *C. pitayensis* and of the Hoja de Lucma and Costrona Fina types of *C. officinalis*.

The ratios or relative proportions of the three principal alkaloids of ninety-two trees of *C. pitayensis* are shown by the position of the ninety-two solid dots in figure 4. It will be noted that nearly all the dots fall in the same zone, the one in which the percentages of both quinine and cinchonine exceed that of cinchonidine. A measure of the variability in alkaloid-forming characteristics of this species is shown by the scatter of the points on the chart. A greater tendency to form cinchonine than quinine may be noted. No sample contained more than 60% of its alkaloids as quinine, while nearly one-third of the samples showed more than 60% of the alkaloids present in the form of cinchonine. No relation of actual total crystallizable alkaloid content of the bark to the position of the dots was noted.

The two types of *C. officinalis* present a contrasting picture, as far as the alkaloid proportions are concerned. In figure 2 it was shown that the Costrona Fina type had essentially the same total crystallizable alkaloid content as the Hoja de Lucma type. The difference in the alkaloids present in the two barks is clearly shown in figure 4. That the Costrona Fina type is primarily a quinine-cinchonidine producer may be seen by the series of triangles along the quinine-cinchonine axis. Relatively few of the samples contained any substantial proportion of cinchonine, and 84% showed more quinine than cinchonidine. Again, there was no detectable relation between total crystallizable alkaloids and the position of the small diagrams on the chart.

The Hoja de Lucma bark contained an average of about equal parts of the two isomers, cinchonine and cinchonidine, according to the approximate analysis shown in table 1. However, it is evident from the data of figure 4 that samples of this type of *C. officinalis* range from high cinchonine and low cinchonidine at the left of the chart to low cincho-

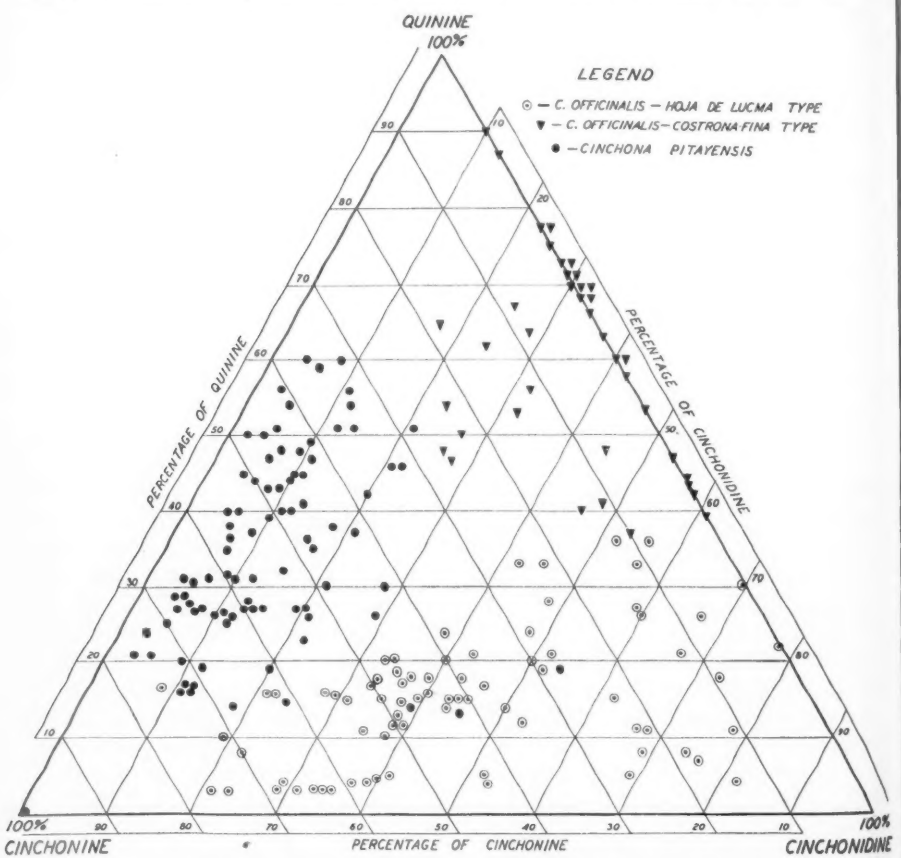


FIG. 4.—Alkaloid ratio diagram for Ecuadoran *C. pitayensis* and two types of *C. officinalis*

nine and high cinchonidine at the right. Very few samples showed any substantial proportion of quinine, and in 82% of the population both cinchonine and cinchonidine exceeded quinine. Whether the Hoja de Lucma is a true *C. officinalis* or a variant type previously described as *C. lucumaefolia* (**5**) is for taxonomists to decide. It does appear that in alkaloid-forming characteristics the two types are distinct.

Another type of alkaloid ratio pattern is presented in figure 5. Here a comparison is made between the Guatemalan Succirubra type and the Ecuadoran Roja type of *C. pubescens*. The Roja points were the same set of commercial samples whose average alkaloid content and variability in composition are shown in table 1 and figure 2.

The Succirubra samples were from a series of twenty-seven individual trees sampled by PLOCHARSKI and STEVENSON in a study of the alkaloid content of Succirubra trees at El Porvenir, Guatemala. Permission from the Foreign Economic Administration to use these unpublished data is gratefully acknowledged.

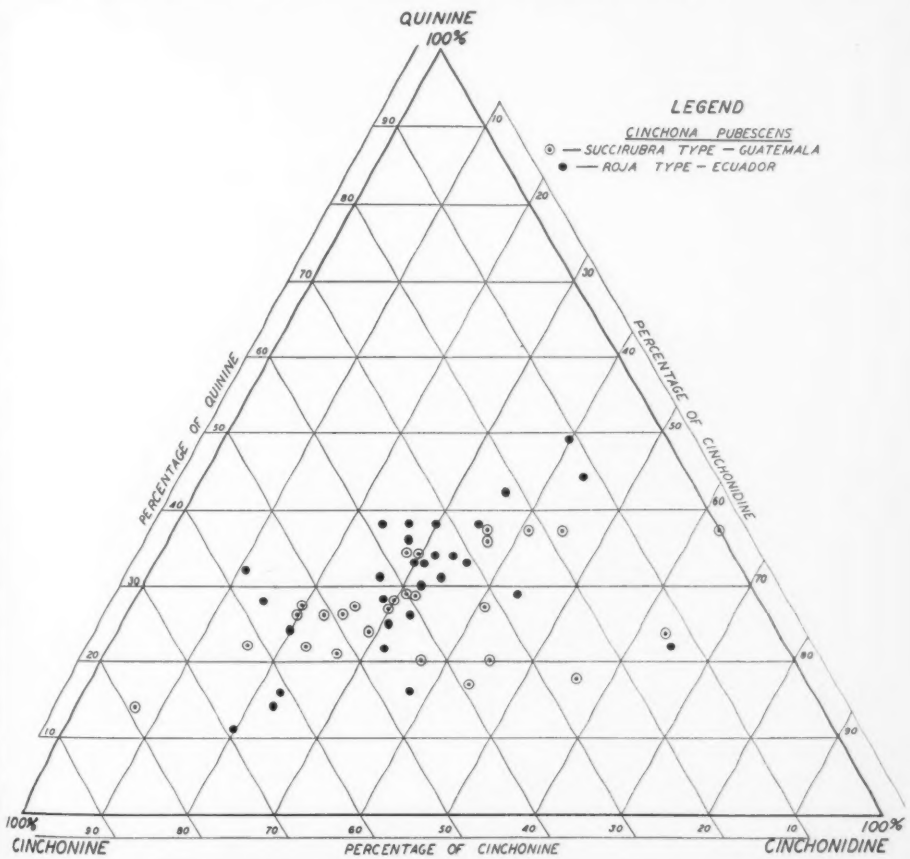


FIG. 5.—Comparison of Ecuadoran Roja with Guatemalan Succirubra type of *C. pubescens*

Both series of points in figure 5 exhibit considerable scatter but tend to group about a general value of 40% cinchonine, 30% cinchonidine, and 30% quinine. The scatter patterns of both types of *C. pubescens* are so similar that they seem to corroborate the record that Succirubra was taken from Ecuador in 1860 (4) to India and returned to the Western Hemisphere in an importation from Ceylon to Guatemala in 1883 (3). No relation between the position of the dots of either series with total crystallizable alkaloid content was found, and the variation indicated by the scatter seems to be probably due to the genetic variability within this type or variety.

A comparison of the Hoja de Lucma type of *C. officinalis* with another Ecuadoran type of similar bark composition occurring in the Baños area of Ecuador is made in figure 6. This latter has been considered a type of *C. officinalis* and shows a very similar scatter pattern when compared with Hoja de Lucma in the diagram. The Baños type tends to have a slightly larger proportion of quinine. Although the Baños type contained an average of 3.96% as compared with only 2.67% of total crystallizable alkaloids in

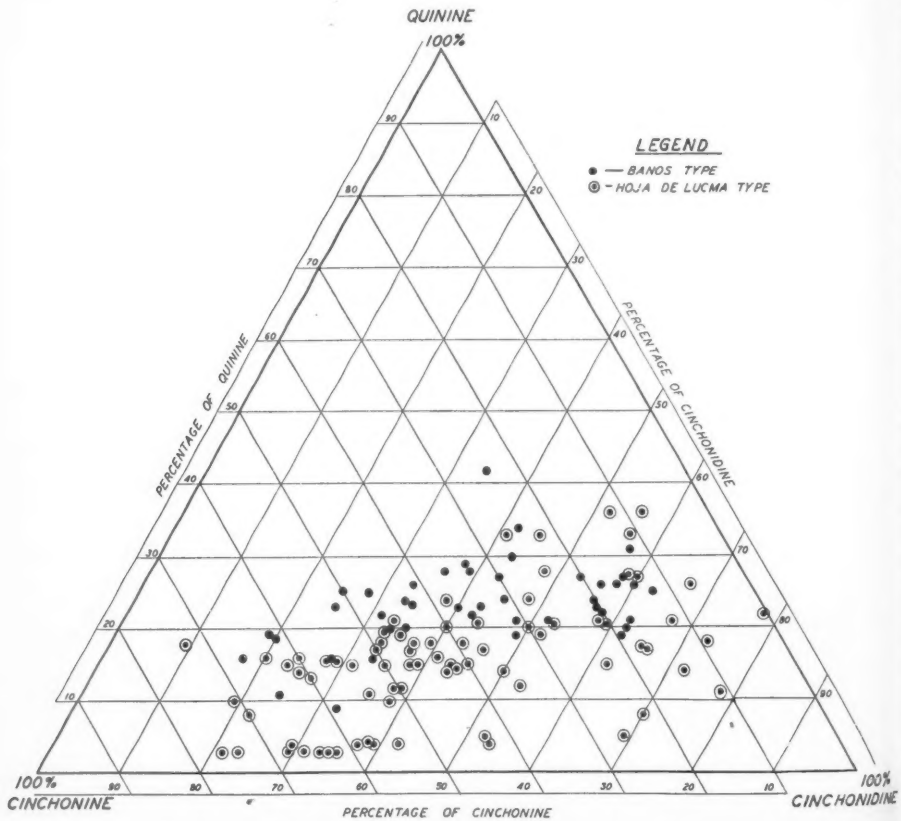


FIG. 6.—Comparison of Hoja de Lucma and Baños types of *C. officinalis*

the Hoja de Lucma, the proportions of the three alkaloids are essentially the same.

Earlier in this paper, the similarity of the proportionate amounts of the alkaloids of the Bolivian Calisaya and Ecuadoran Costrona Fina types of *C. officinalis* was pointed out. As may be seen from figure 7, both are predominantly quinine-formers, and scatter patterns tend to overlap considerably, in spite of the fact that the Calisaya contains nearly twice the total crystallizable alkaloids found in samples of Ecuadoran Costrona Fina.

It may be noted that cinchonine was entirely absent in many of the Ecuadoran Costrona Fina samples, while in every Calisaya sample at least a small proportion was present. Both can be classified as quinine barks, in marked contrast with the other types of cinchona shown in the preceding two diagrams.

The data in the diagrams already presented have been taken principally from commercial samples. As such, they show the general characteristics of the species and type but do not present so complete a picture of genetic variation as may be

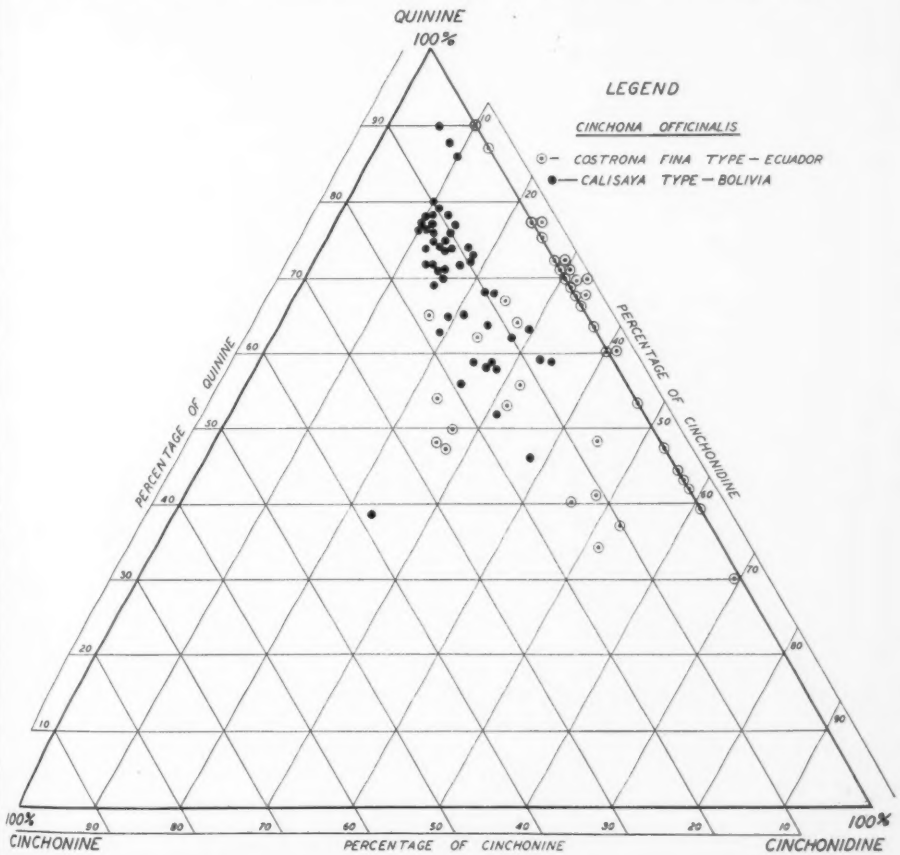


FIG. 7.—Comparison of Bolivian Calisaya with Ecuadoran Costrona Fina types of *C. officinalis*

obtained from a study of individual tree samples. Variation of the latter type is shown in figure 8. In this chart are presented scatter diagrams from samples of the fifty-seven individual trees of *C. pitayensis* sampled in Ecuador and from sixty of the Ledgeriana trees sampled at the Punizas planting in Peru. The twenty Ledgeriana trees containing appreciable amounts of quinidine were not included, as no practical way has been found to plot four variables simultaneously.

The *C. pitayensis* points occur in two distinct zones in figure 8. A group of nine of the fifty-seven samples containing nothing but cinchonine, and less than 2% total crystallizable alkaloids, rests at the cinchonine corner of the triangle. The remaining forty-eight samples occupy almost exactly the same zone of the chart as occupied by the commercial field samples shown in figure 4. The cinchonine samples seem definitely apart from the *C. pitayensis* type and might represent trees of another species, such as *C. pubescens* Bofuda, known to occur at 2000-feet lower elevation on the same mountain. On the other hand, the Bofuda

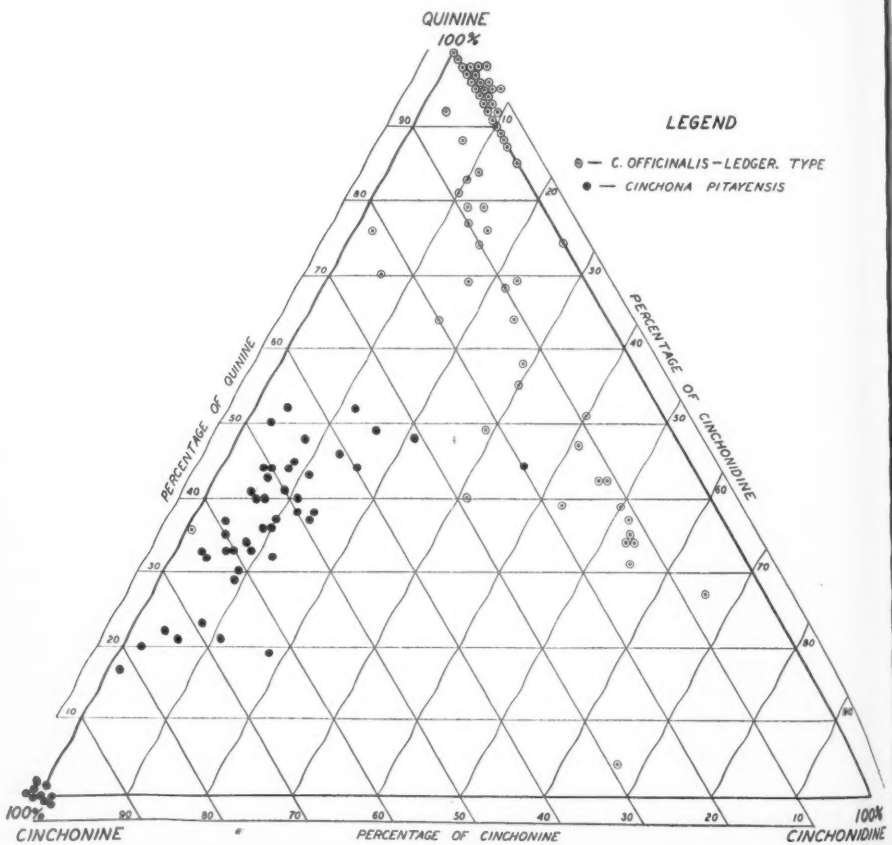


FIG. 8.—Alkaloid ratio pattern of individual tree populations of *C. pitayensis* and cultivated Ledgeriana trees of *C. officinalis*.

type is so distinct from *C. pitayensis* that it could hardly have been mistaken at the time leafy cuttings were taken from each tree for propagation. A possibility exists that these cinchonine trees may be the result of segregation of a supposedly pure species, or else the result of the production of hybrid seed many years ago by the pollination of the *C. pitayensis* trees by *C. pubescens* pollen carried up the mountain several miles by insects or wind.

The fact that Ledgeriana is primarily a quinine-producer is well illustrated in figure 8, which shows a heavy cluster of points above the 85% quinine level. Most of the remaining points occupy the zone containing in figure 7 most of the Bolivian Calisaya samples. The scatter pattern for this Ledgeriana population may well represent the segregation of seedlings derived from plants originally selected in Java for quinine production. The few trees of low quinine and high cinchonine or cinchonidine content may be taken to represent extremes in genetic variability and reversion to a parent type of very different alkaloid-producing characteristics. The position of the points was not found to be related to leaf type, tree age, or any other discernible characteristic. It is of interest that the points not representing trees of predominant (above 80%) quinine production occur in almost the same zone of the chart as samples of Bolivian Calisaya, reported to be the parent material from which Ledgeriana was selected (**6**).

Summary

1. Data are presented describing the geographic distribution and showing the approximate composition of the principal commercial cinchona barks of Ecuador, together with comparative composition of other American barks.

2. While the alkaloid quinine is often thought of as the common constituent of cinchona bark, the three additional alkaloids—quinidine, cinchonine, and cinchonidine—are commonly present. The latter two are often present in Ecuadoran barks in amounts greatly exceeding quinine.

3. A study has been made of the variability in composition of Ecuadoran and other American cinchona barks in order to determine their value as breeding material or as sources for the selection and development of clones of high alkaloid-producing capacity.

4. Alkaloid ratio diagrams for the principal cinchona types are presented to show the relative composition of a bark with respect to its alkaloid constituents. These diagrams appear to offer a simple graphic method of presenting genetic variability within a population and may be used as an aid in variety or species delimitation.

Estación Experimental del Ecuador and
Foreign Economic Administration
Quito, Ecuador

LITERATURE CITED

1. Martin, William E., and Crandall, Bowen, Observations on culture and plant disease in the Punizas cinchona planting and an evaluation as seed source and source of propagating material. Colonias y Forests, Ano 1, 4–15. Lima, Peru. 1944.
2. McDermott, John J., and Crandall, Bowen, Punizas, an American source of cinchona planting stocks. (In press.)
3. Rosengarten, Fritz, History of the cinchona project of Merck & Co., and Experimental Plantations Inc. Merck & Co., Rahway, N.J. Pp. 45. 1944.
4. Spruce, Richard, Notes of a botanist on the Amazon and Andes. Macmillan, London. 1908.
5. Standley, P. C., Rubiaceae of Ecuador. Publ., Field Mus. Nat. Hist., Bot. Series. 7:(2) 179–251. 1931.
6. ———, Rubiaceae of Bolivia. Publ. Field Mus. Nat. Hist., Bot. Series. 7:(3) 255–339. 1931.
7. Supplement to Pharmacopoeia of the United States. 12th ed. 1944.

HISTOLOGICAL REACTIONS OF BEAN PLANTS TO CERTAIN OF THE SUBSTITUTED PHENOXY COMPOUNDS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 569

J. M. BEAL

Introduction

This paper is one of a series, begun in 1936 (6), dealing with the histological reactions of plants to various growth-regulating substances. The reactions of a number of plants have been described, but no detailed studies have been reported on the histological reactions of any plant to applications of the substituted phenoxy compounds. The gross telemorphic effects of certain of these compounds have been recorded in earlier papers (1, 2). The results there reported accorded with some of those previously given by ZIMMERMAN (10) and by ZIMMERMAN and HITCHCOCK (11). Strikingly different responses or effects have been induced by different phenoxy compounds. In addition, it has been shown (2) that the carrier in which a specific compound is applied may profoundly influence the character and degree of the response. It was demonstrated, for example, that 2-chlorophenoxyacetic acid was effective when applied in Carbowax but largely ineffective in lanolin. An almost completely opposite effect resulted from the application of 4-chlorophenoxyacetic acid in either of the two carriers. Both carriers were almost equally effective for 2,4-di- and 2,4,5-trichlorophenoxyacetic acids, but with somewhat more rapid growth responses and form changes when Carbowax was the carrier. When either of the latter two compounds was applied at a 1% concentration in Carbowax, the majority of the treated plants were dead within 3 weeks. The surviving plants recovered slowly and then developed chiefly small misshapen leaves. Although slightly fewer plants were killed when lanolin was the carrier, there was little other difference in the responses.

Because the form changes and growth responses had been so pronounced from the application of 1% mixtures of the phenoxy compounds at the bases of the blades of the nearly full-sized heart-shaped leaves of the bean, it was decided to conduct further experiments and apply the same compounds at 0.5% concentration. Three additional series of beans have been grown in flats, under conditions which simulated as closely as possible those of the earlier experiments, and treated at different seasons. The first was started on March 1, 1945, and treated on March 13. Temperatures (although fairly cool) and light conditions were favorable to growth at the time. These plants grew well but responded more slowly than those to which 1% mixtures had been applied at approximately the same period the preceding year, when light and temperature conditions were similar. A second series was planted on June 10 and treated on June 21, when the day temperatures in the greenhouse were higher than during March and April. The responses were greater than in the first series but were still somewhat slower than following application of the 1% mixtures. A third series planted on July 20 was treated on July 28. Just

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

before and immediately after treatment of this series, the outside temperatures exceeded 90° F. during the day and the relative humidity was high. As a consequence, the Carbowax mixtures absorbed water and liquefied, often spreading along the surface of the leaf or down the petiole. The responses in this series were as rapid and as pronounced as they had been with the 1% mixtures, and approximately the same percentage of plants were killed. In general, the growth effects were essentially similar to those previously reported (**2**), with the least responses being induced by applications of 2-chlorophenoxyacetic acid.

Recently, 2,4-dichlorophenoxyacetic acid has come into prominence as a differential herbicide (**3, 7, 8, 9**), promising to become highly important for this purpose. Only one report has appeared which deals with the histological changes resulting from application of this substance. TUKEY *et al.* (**9**) treated bindweed and sow thistle with aqueous sprays of 2,4-dichlorophenoxyacetic acid at 1000 p.p.m. in 0.5% Carbowax 1500, applied during midsummer while the plants were growing vigorously. This resulted in greatly increased cell division in all cambial zones and phloem regions of the stem and rhizome of bindweed, with enlargement and rupture of cortical cells of the rhizome. The root responded similarly but more slowly. In the rhizome of sow thistle, enlargement and tearing of cortical cells was frequent, accompanied by rupture of the periderm. The cambial zone and phloem regions showed disorganized, large-scale cell division. The investigators suggest that the increase in meristematic activity indicates an increase in the rate of respiration, which would deplete the food reserves.

For the present histological studies, usually the entire second internode (seldom exceeding $\frac{1}{4}$ inch in length), a segment of the first internode just below the second node, and a segment of the hypocotyl just below the cotyledonary node were fixed in Navashin's solution, handled according to the tertiary butyl-alcohol paraffin method, and sectioned at 10μ.

Observations

As reported previously (**2**), the place or region of response is highly variable, not only for the different phenoxy compounds but also for any one of them. Usually little indication of cell activity as a result of treatment is evident in any portion of the stem until approximately 48 hours have elapsed (figs. 2*B*, 3*A*, *B*). Sometimes, however, activity begins earlier in the second internode (fig. 2*A*), although this is not the general occurrence. The time required is not surprising when it is recalled that the point of application of the mixture is $1\frac{1}{2}$–2 inches distant from the top of the first internode and that the chemical, or some derivative from it, must travel this distance and then up or down the stem before noticeable effects can be produced on the cells above and below the second node.

Although most of the present studies have been made on the first internode, sections from the second internode and the hypocotyl have also been investigated and photographed.

At the time of treatment, the second internode had scarcely begun to elongate, being in most cases only 3–5 mm. in length. The trifoliate leaf was as yet unexpanded. Transections show fascicular cambium, however, which has begun to cut off secondary xylem and phloem, but the interfascicular cambium is not complete (fig. 1*A*).

The second internode was usually little

affected by applications of 2-chlorophenoxyacetic acid, elongation apparently occurring normally with usually no swelling. Following application of 4-chlorophenoxyacetic acid, however, the second internode became much swollen, pale yellow in color, and failed to elongate in approximately one-third of the plants. Death of these swollen internodes usually occurred in less than 3 weeks following treatment, but lateral shoots commonly developed on these plants from the buds in the axils of the heart-shaped leaves, as well as occasionally from those in the axils of the cotyledons. The development of these shoots was slow, and they produced small, narrow, and usually rolled leaflets (2). As a result of cell proliferation, however, the internode frequently becomes swollen in all the tissues except the epidermis, localized areas of the outer cortex, and perhaps the pericycle (figs. 2*A*, 4*A*). The inner cortex, the endodermis, phloem and xylem parenchyma, cambium, ray parenchyma, and pith all show marked activity, with the greatest activity in the cambium, phloem, and ray parenchyma. It has not been possible with the sections available to determine the degree of activity in the pericycle, but it appears unresponsive.

The tissue responses shown in figure 4*A* are similar to those of figure 14 in the paper by KRAUS, BROWN, and HAMNER (6), which deals with the effects of indoleacetic acid applied to the distal end of decapitated second internodes of bean. One rather striking difference is that root primordia fail to develop in the second internodes of any of the plants following application of the phenoxy compounds at the bases of the blades of the heart-shaped leaves (fig. 10), even after as long as 20 days (fig. 13). It should be pointed out that the degree of maturity of the second internode at the time of treatment is different in the two types of experiments. In the plants to which the compounds are applied at the base of the heart-shaped leaves, the chemical—or some derivative from it—must travel down the petiole and then upward into the second internode before it can incite reaction there. In experiments with decapitated plants, the most striking responses have generally occurred just below the place of application of the chemicals. This may have some bearing on the development or the failure of development of roots following the two methods of application.

The second internode seldom elongates appreciably following treatment with 2,4-di- or 2,4,5-trichlorophenoxyacetic acids but commonly develops the same type of swelling as that resulting from 4-chlorophenoxyacetic acid. Microscopic sections show the same tissues activated and the rate of development to be almost identical from the three compounds.

The tissue responses in the first internode, while much more general and pronounced following applications of 2,4-di- and 2,4,5-trichlorophenoxyacetic acids, were nevertheless similar from all the compounds employed, with the least responses induced by the 4-chlorophenoxyacetic acid. Little indication of increased meristematic activity was evident in the first internode, as was usually true of the second, until approximately 48 hours after treatment (figs. 2*B*, 3*A*, *B*). These early responses are limited to division of the cells of the endodermis, cambium, and medullary rays, with no signs of activation of cells in the other tissues. The early divisions are all in a tangential plane; some of the later ones are radial. Comparison of these figures with figure 1*B* (from a section of a control plant fixed at the time of treatment)

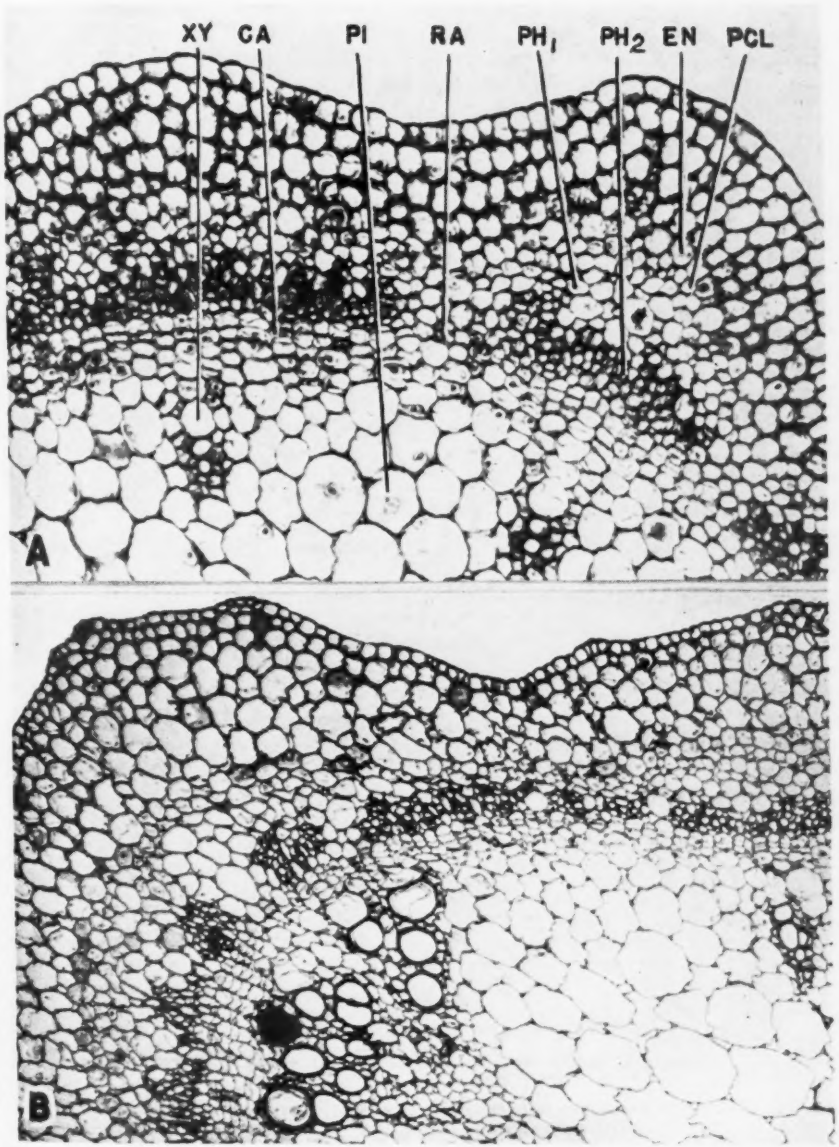


FIG. 1.—*A*, transection of second internode of bean at time of treatments, showing various tissues and stage of development. (*xy*, xylem; *ca*, cambium; *pi*, pith; *ra*, ray; ph_1, primary phloem; ph_2, secondary phloem; *en*, endodermis; *pcl*, pericycle.) *B*, first internode of same plant, showing continuous ring of cambium; some derivatives of cambium essentially mature.

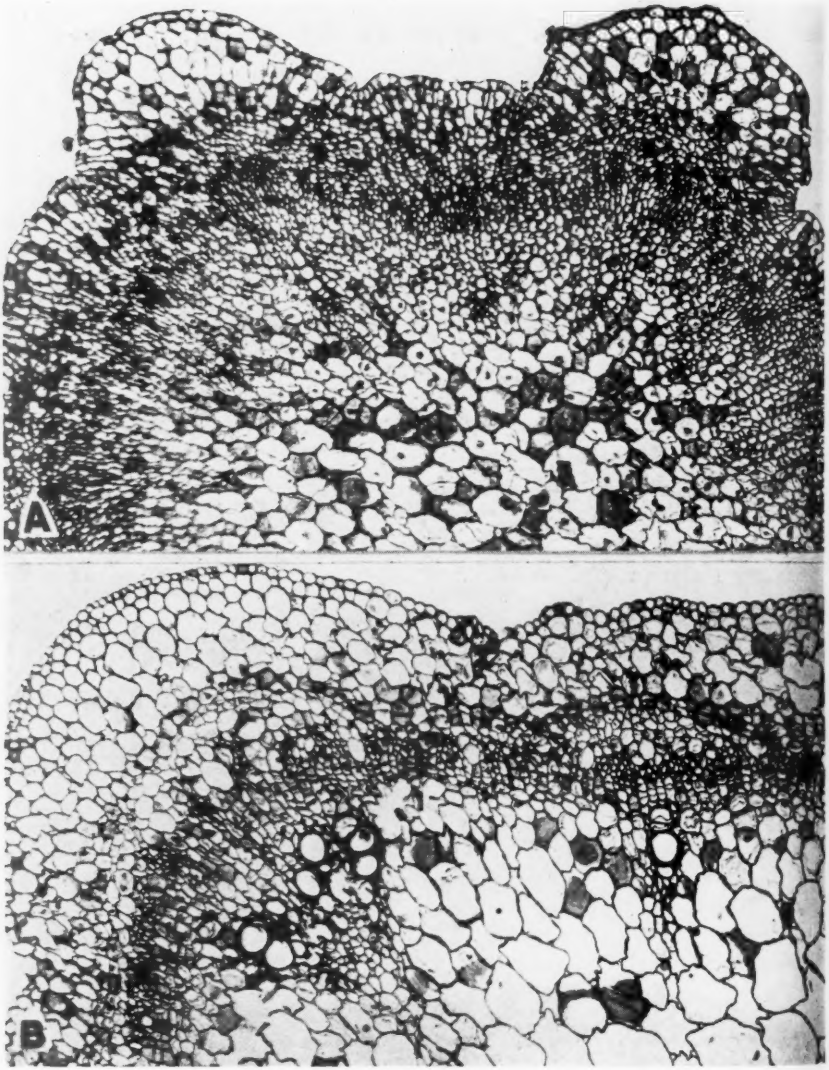


FIG. 2.—Forty-eight hours after treatment with 2,4-dichlorophenoxyacetic acid in Carbowax: *A*, second internode, showing marked activity in all tissues except epidermis, possibly pericycle, and portions of outer cortex. *B*, first internode, showing activity only in endodermis, cambium, phloem, and ray parenchyma; principal activity in endodermis and cambium.

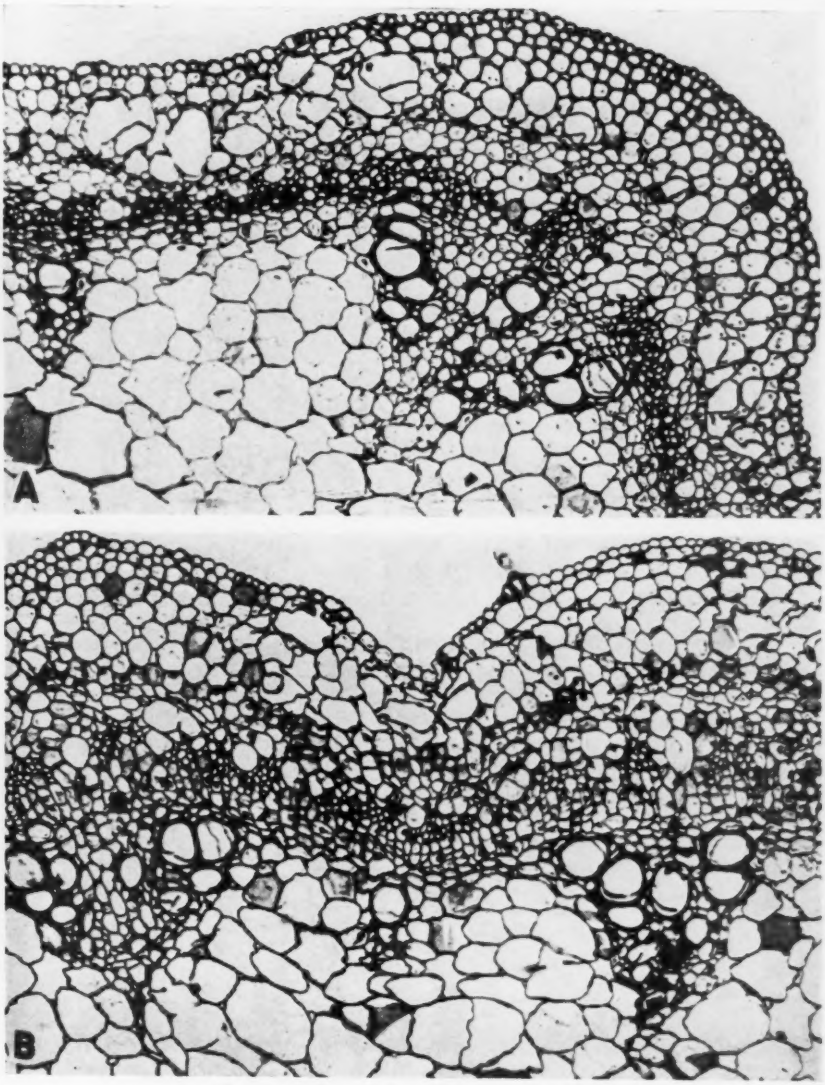


FIG. 3.—First internode 48 hours after treatment with 2,4,5-trichlorophenoxyacetic acid: *A*, in Carbowax, showing practically no induced activity; and *B*, in lanolin, showing activity beginning in endodermis, cambium, and ray parenchyma, with slight activity in phloem parenchyma. Pericycle and pith apparently unaffected.

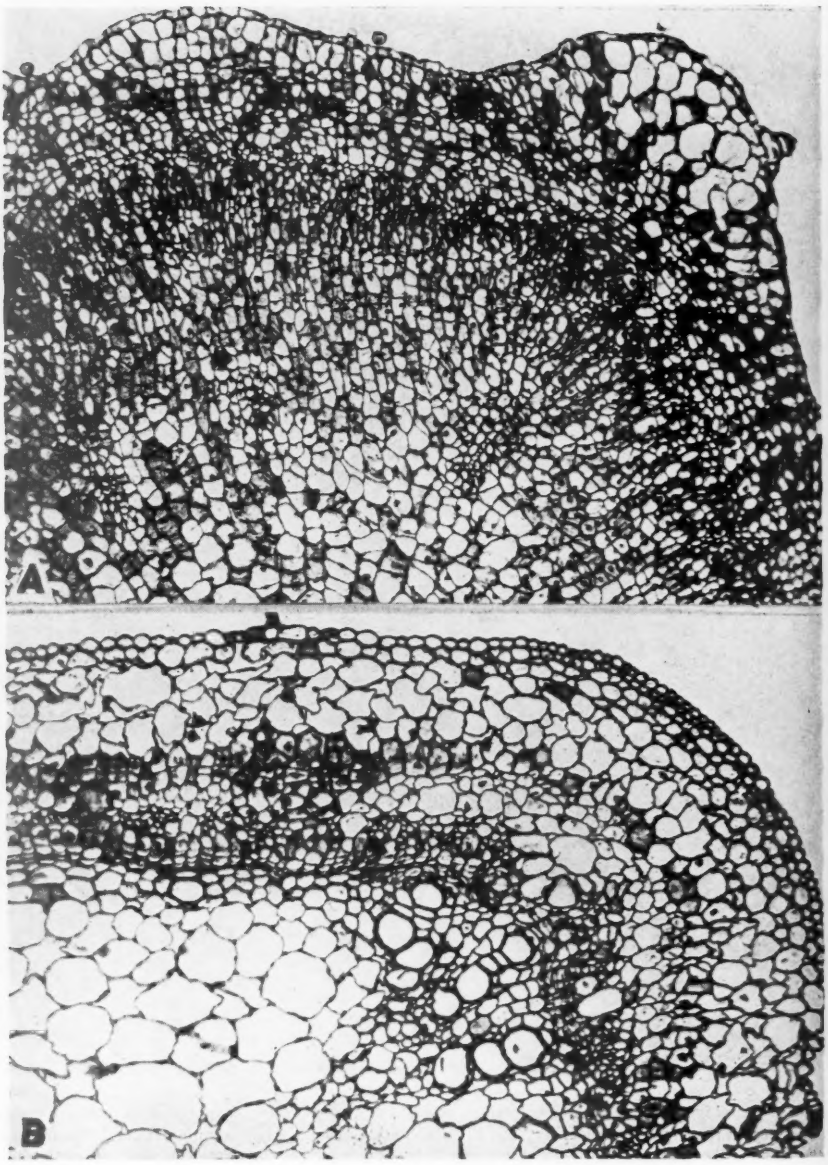


FIG. 4.—Seventy-two hours after treatment: *A*, second internode after treatment with 4-chlorophenoxyacetic acid in lanolin. All tissues except epidermis and pericycle have been active; similar to fig. 2*A* but responses now more extensive. *B*, first internode after treatment with 2,4-dichlorophenoxyacetic acid in Carbowax. Activity principally in endodermis, cambium, and ray parenchyma. Pericycle and pith unaffected.

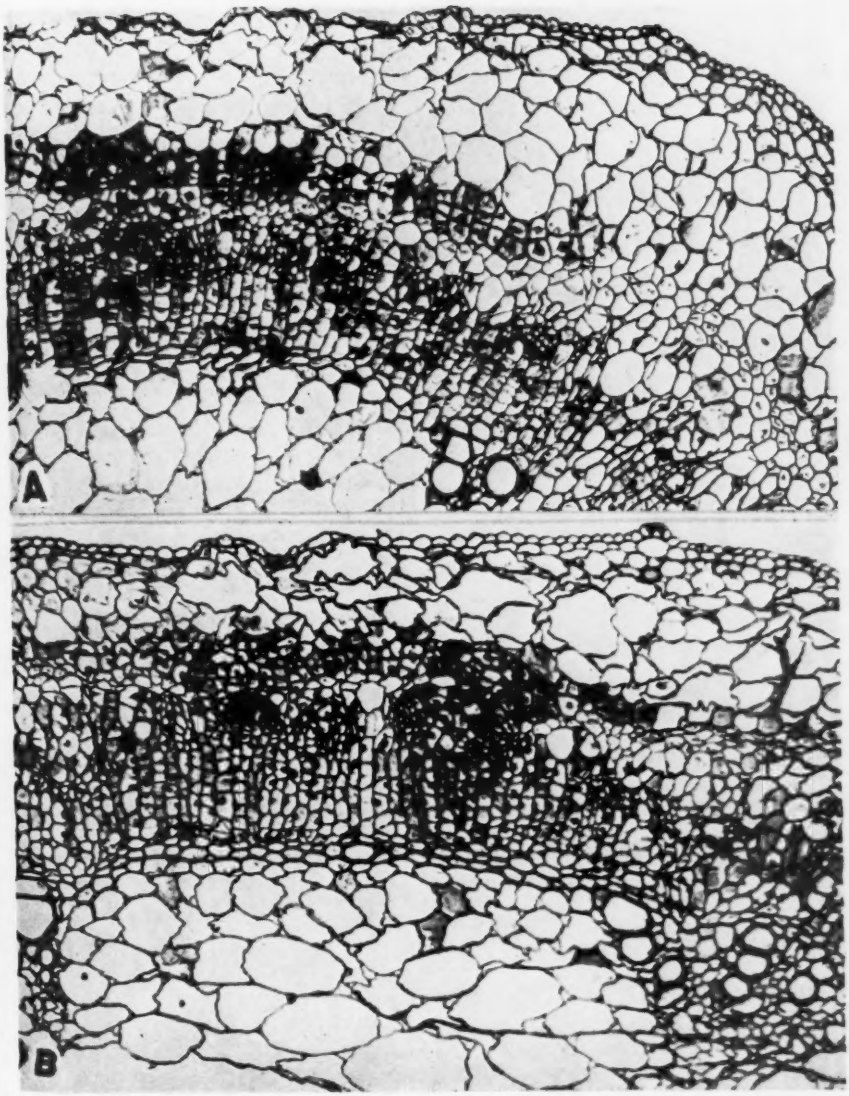


FIG. 5.—First internode 72 hours after treatment with 2,4,5-trichlorophenoxyacetic acid: *A*, in Carbowax; and *B*, in lanolin. Responses similar in both, with greatly increased activity in endodermis, cambium, phloem, and ray parenchyma.

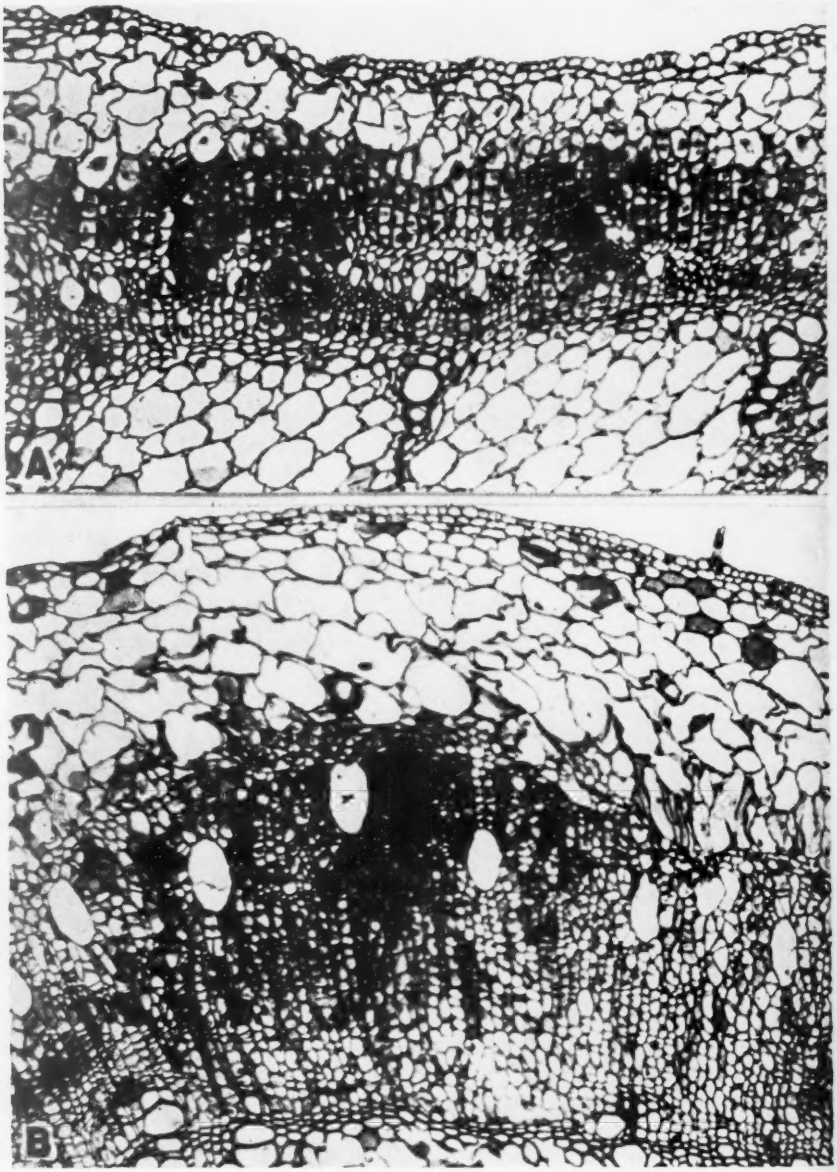


FIG. 6.—*A*, first internode 80 hours after treatment with 4-chlorophenoxyacetic acid in lanolin. Principal activity in endodermis and ray parenchyma, with less activity in cambium and phloem parenchyma. *B*, upper portion of hypocotyl 96 hours after treatment with 2,4-dichlorophenoxyacetic acid in lanolin, showing root primordium derived chiefly from cambium, phloem, and ray parenchyma. Endodermis showing little or no activity.

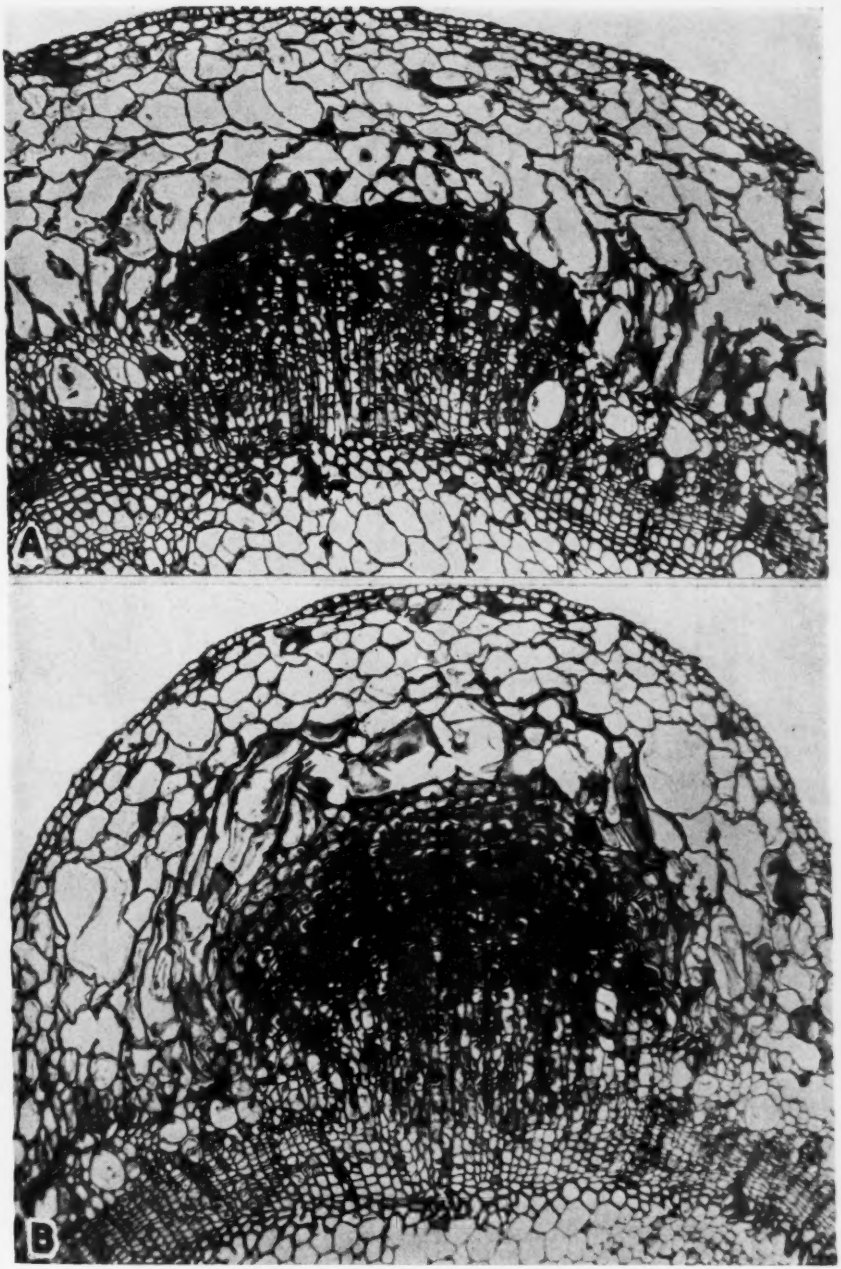


FIG. 7.—Upper portion of hypocotyl: *A*, 96 hours after treatment with 2,4,5-trichlorophenoxyacetic acid in lanolin; and *B*, 144 hours after treatment with 4-chlorophenoxyacetic acid in lanolin. Root primordia organized chiefly from phloem and ray parenchyma, with less activity in cambium. Endodermal and some of inner cortical cells enlarged and crushed over end of root primordium.

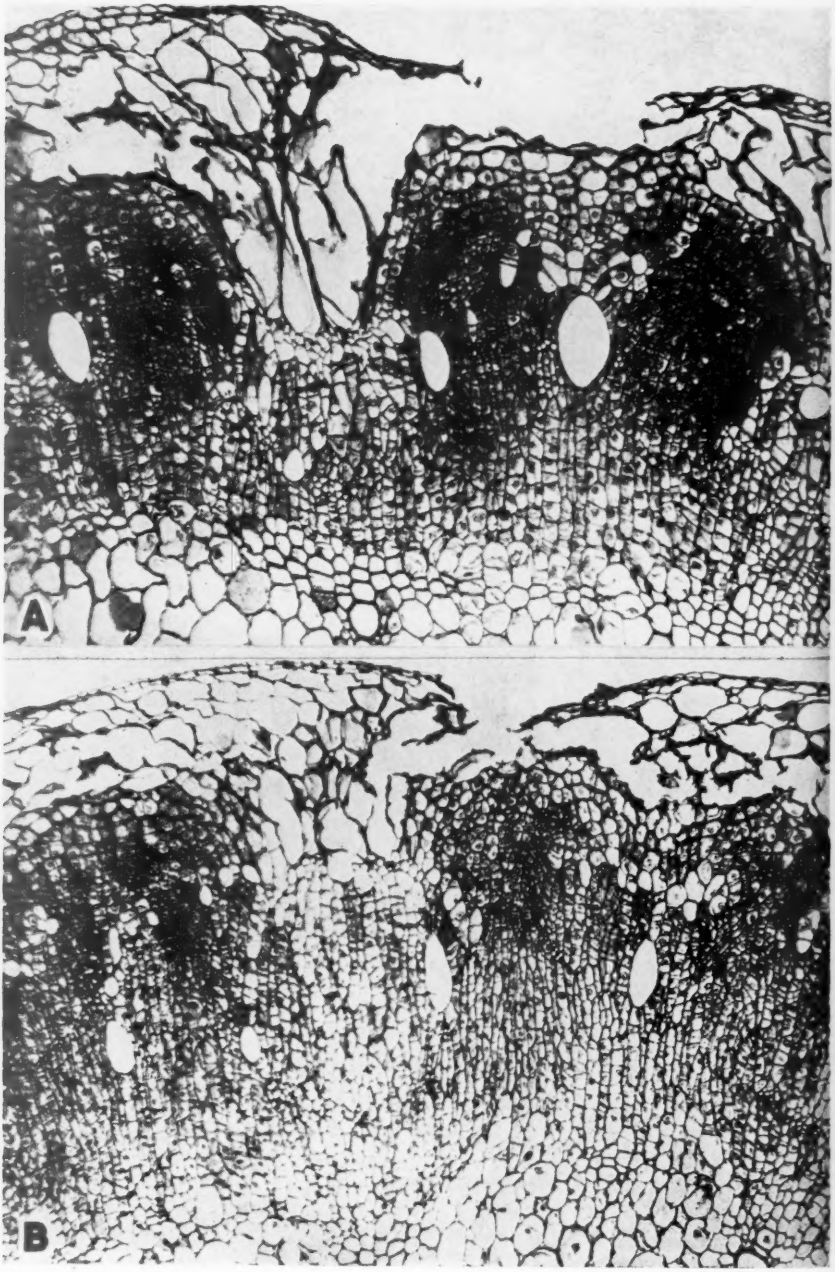


FIG. 8.—First internode 120 hours after treatment with 2,4-dichlorophenoxyacetic acid: *A*, in Carbowax; and *B*, in lanolin. Same tissues active as in fig. 6*B*.

FIG. 9.—*A*, first internode 120 hours after treatment with 2,4,5-trichlorophenoxyacetic acid in Carbowax. Tissues activated and responses similar to those in fig. 8*A*, *B*. *B*, first internode 192 hours after treatment with same compound applied in lanolin. Central core of adventitious roots derived from ray, outer portions from phloem, and tips capped with primary phloem and its derivatives. Endodermis and cortex chiefly crushed and dead.

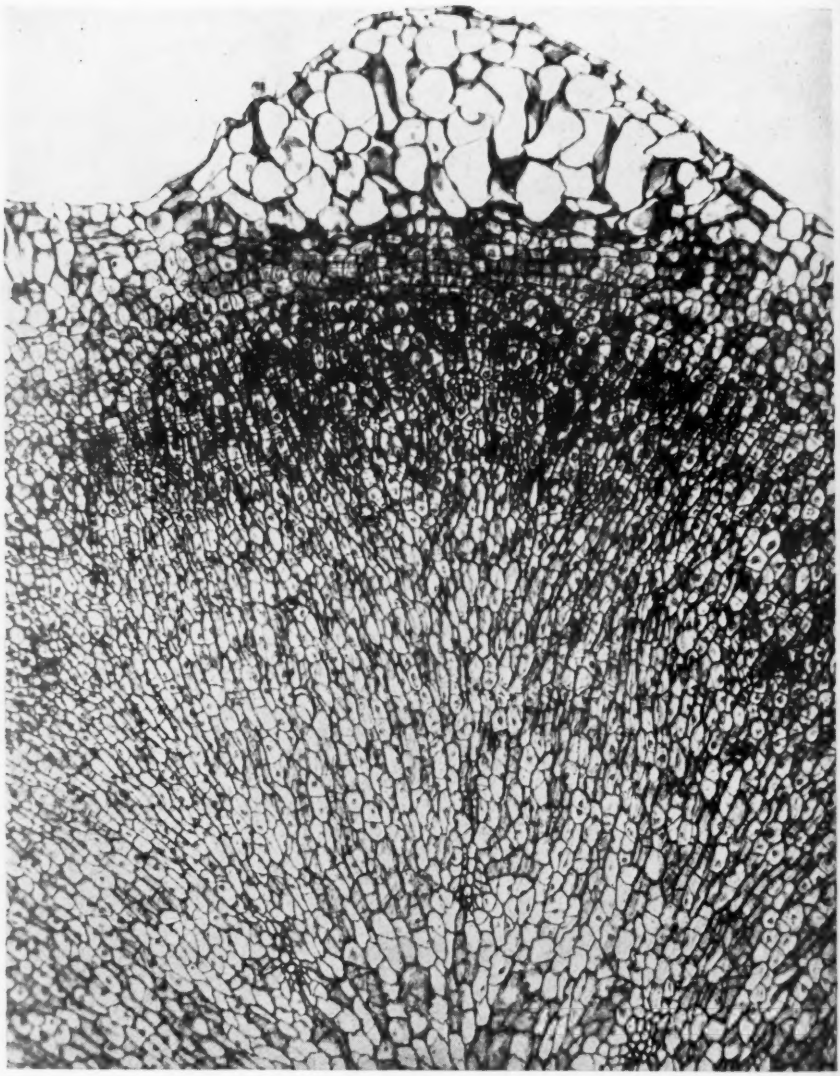


FIG. 10.—Second internode just above second node 144 hours after treatment with 4-chlorophenoxyacetic acid in lanolin, showing marked activity in all tissues except epidermis (and possibly pericycle). Cambium especially active.

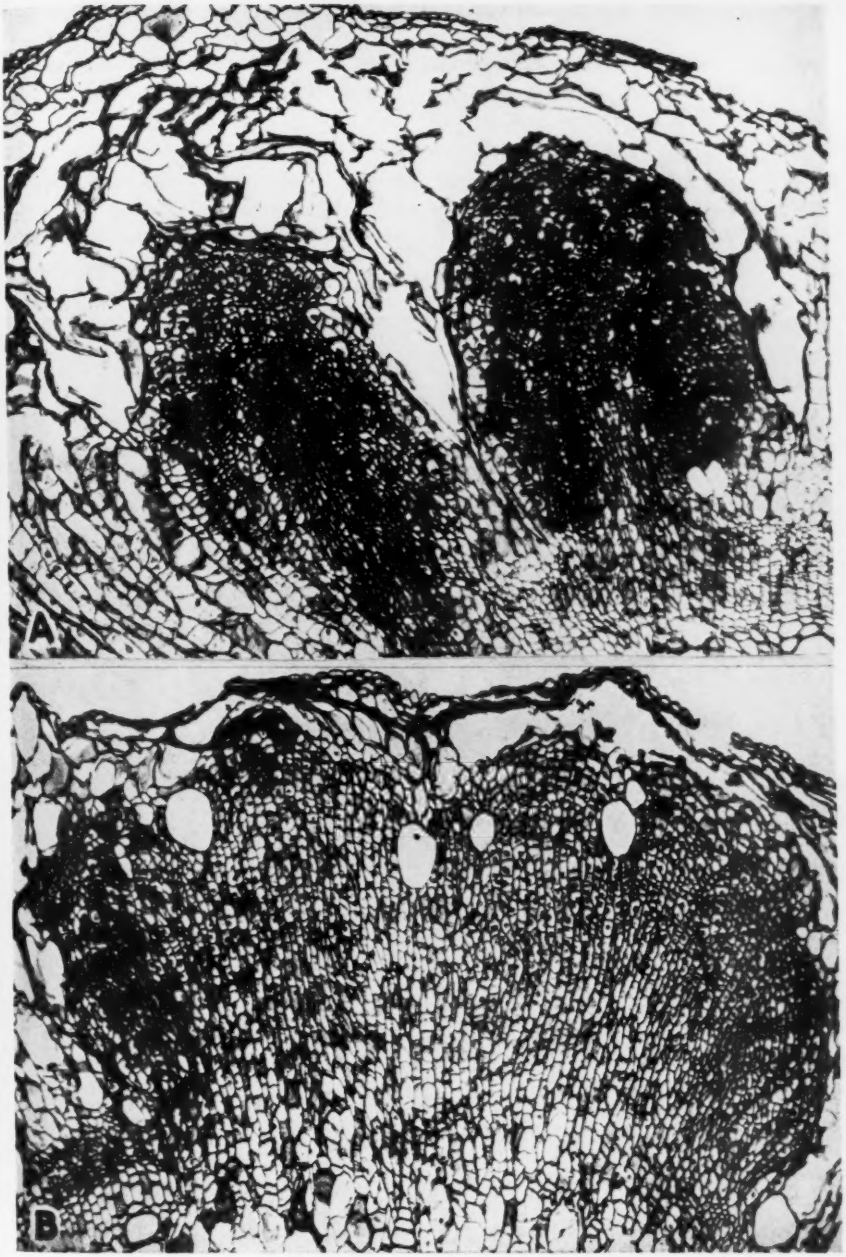


FIG. 11.—First internode 240 hours after treatment with 2,4-dichlorophenoxyacetic acid: *A*, in Carbowax; and *B*, in lanolin. Activities similar to but more advanced than in fig. 8*A*, *B*. Epidermis and cortex mostly dead and broken over root primordia.

brings out certain striking differences. In the control (fig. 1*B*), while a complete ring of cambium is present and an appreciable amount of secondary phloem and xylem have differentiated, the endodermis, ray, and phloem parenchyma show no unusual activation (figs. 2*B*, 3*A*, *B*).

At 72–80 hours after treatment the rate of division increases in the endodermis, cambium, and ray tissues, with some divisions having taken place in the phloem parenchyma (figs. 4*B*, 5*A*, *B*, 6*A*). The most highly reactive tissues are the ray and phloem parenchyma, however, and it is principally from them

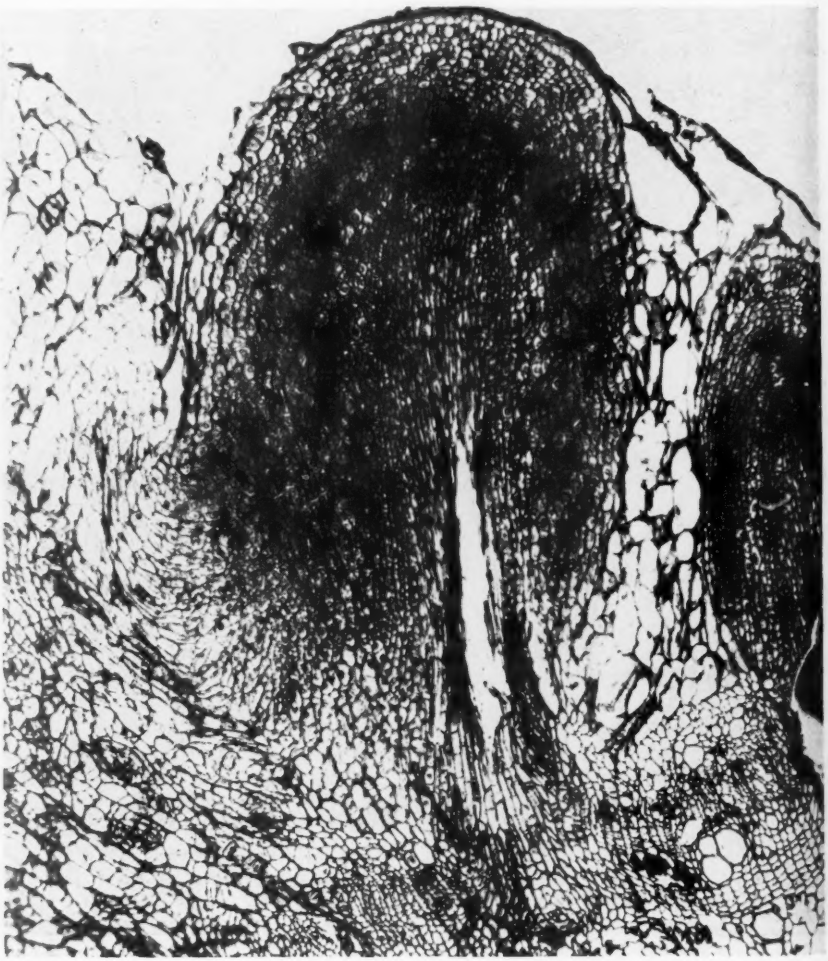


FIG. 12.—First internode 480 hours after treatment with 4-chlorophenoxyacetic acid in Carbowax. Relation of roots to rays similar to that in fig. 9*B*. Cortical tissues mostly dead and crushed.

and their derivatives that the root primordia originate. Concurrently, the cambium, both fascicular and interfascicular, becomes active. The pericycle appears to be inactive and not involved in any of these changes.

At 120 hours, root primordia begin to show definite organization (figs. 8*A*, *B*, 9*A*). In some stems the endodermis fails to become active or there are so few divisions that it plays no significant part in root formation. And while the cambium and its derivatives continue to divide, they contribute less to the formation of the root primordia than do the ray and phloem parenchyma. Cells of the pericycle have in general remained inactive, unlike *Iresine* (5) and *Mirabilis* (4), in both of which they are highly responsive. Well-developed roots are present in some stems at 192 hours after treatment (fig. 9*B*), although in most stems such roots are not present until 10 days (fig. 11*A*, *B*) or more have elapsed (fig. 12). In these latter figures the epidermis and outer cortical and endodermal cells are mostly dead and collapsed. Perhaps a few derivatives of the endodermis are still alive over the distal ends of the root primordia, but most of this tissue consists of primary phloem. The induced roots originate in the pith rays and may occur in one or more of them.

Activity in the hypocotyl, which re-

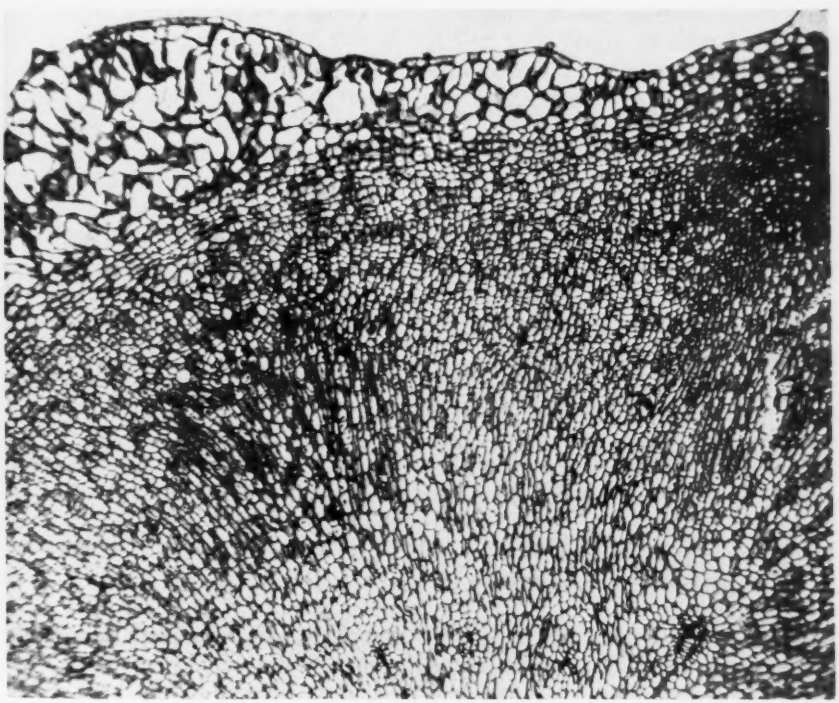


FIG. 13.—Second internode just above node of heart-shaped leaf 480 hours after treatment with 4-chlorophenoxyacetic acid in lanolin. All tissues, especially cambium, have proliferated actively except epidermis, pericycle, and localized areas of outer cortex, but no roots have differentiated.

sults in swelling or in root formation, or both, is common following treatment with 2,4-di- and 2,4,5-trichlorophenoxyacetic acids. The swelling and subsequent root development commonly involve most of the length of the hypocotyl. And although comparatively few plants show responses in the hypocotyls to applications of 2- and 4-chlorophenoxyacetic acids, and then chiefly in localized areas, the histological responses resulting from the four chemicals are essentially identical and involve the same tissues and tissue systems (figs. 6*B*, 7*A*, *B*). The greatest activity occurs in the ray and phloem parenchyma, with somewhat less in the cambium. The pericycle is inactive, or only slightly active, and plays a very minor role in root development. Root production is largely restricted to the angles of the hypocotyl distal to the protoxylem points of the main or primary vascular bundles.

From an examination of the foregoing figures it is evident that, when the substituted phenoxy compounds are applied at the bases of the heart-shaped leaves of the bean, the tissue responses in the second internode are strikingly similar to those induced by a number of other compounds when such compounds are applied to the cut surface of the young second internode which has been decapitated just below the first trifoliate leaf. Surprisingly, however, no roots developed in this internode following application of the phenoxy compounds to the bases of the heart-shaped leaves, even after periods as long as 20 days following application.

In the first internode and hypocotyl the responses are much more restricted than in the second internode. In the first two of these, generally only the endodermis, cambium, phloem, and ray parenchyma respond. From the cambium, phloem, and ray parenchyma, especially the phloem and ray parenchyma, adventitious roots develop in abundance. Perhaps the greater maturity of the cells in the tissues of the first internode and hypocotyl as compared with the same tissues in the second internode at the time of treatment is, in part at least, responsible for the difference in responses.

Summary

1. Four substituted phenoxy compounds have been applied at the bases of the nearly full-sized heart-shaped leaves of the bean at 0.5% concentration, using both Carbowax 1500 and lanolin as carriers. Lanolin was ineffective as a carrier for 2-chlorophenoxyacetic acid and Carbowax was ineffective for 4-chlorophenoxyacetic acid. The gross responses of the plants to the weaker concentrations were almost identical with those reported previously (**2**).

2. Histological studies were made of material collected and preserved at 12-hour intervals up to a total of 480 hours. Certain marked differences in responses were observed as between the second internode on the one hand and the first internode on the other. There were also differences in the degree of responses of the plants to the four compounds, although 2,4-di- and 2,4,5-trichlorophenoxyacetic acids resulted in strikingly similar responses.

3. The second internode showed little or no histological response to 2-chlorophenoxyacetic acid. When treated with the three other compounds it became much swollen and generally failed to elongate appreciably in a high percentage of the plants. With the exception of the epidermis, possibly the pericycle,

and limited portions of the outer cortex, all the other tissues responded actively; but no adventitious roots developed, even after 20 days following treatment.

4. The histological responses in the first internode and hypocotyl were strikingly similar from all four compounds, but—in contrast to the second internode—only the endodermis, cambium, phloem, and ray parenchyma were activated. Roots developed in abundance.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BEAL, J. M., Some telemorphic effects induced in sweet pea by application of 4-chlorophenoxyacetic acid. BOT. GAZ. **105**:471–474. 1944.
2. ———, Further observations on the telemorphic effects of certain growth-regulating substances. BOT. GAZ. **106**:165–178. 1944.
3. HAMNER, C. L., and TUKEY, H. B., Selective herbicidal action of midsummer and fall applications of 2,4-dichlorophenoxyacetic acid BOT. GAZ. **106**:232–245. 1944.
4. HAMNER, K. C., Histological responses of *Mirabilis jalapa* to indoleacetic acid. BOT. GAZ. **99**:912–954. 1938.
5. HARRISON, B. F., Histological responses of *Iresine lindenii* to indoleacetic acid. BOT. GAZ. **99**: 301–338. 1937.
6. KRAUS, E. J., BROWN, NELLIE A., and HAMNER, K. C., Histological responses of bean plants to indoleacetic acid. BOT. GAZ. **98**:370–420. 1936.
7. MARTH, P. C., and DAVIS, F. F., Relation of temperature to the selective herbicidal effects of 2,4-dichlorophenoxyacetic acid. BOT. GAZ. **106**:463–472. 1945.
8. MARTH, P. C., and MITCHELL, J. W., 2,4-Dichlorophenoxyacetic acid as a differential herbicide. BOT. GAZ. **106**:224–232. 1944.
9. TUKEY, H. B., HAMNER, C. L., and IMHOFE, BARBARA, Histological changes in bindweed and sow thistle following applications of 2,4-dichlorophenoxyacetic acid in herbicidal concentrations. BOT. GAZ. **107**:62–73. 1945.
10. ZIMMERMAN, P. W., The formative influences and comparative effectiveness of various plant hormone-like compounds. Torreya **43**:98–115. 1943.
11. ZIMMERMAN, P. W., and HITCHCOCK, A. E., Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. Contrib. Boyce Thompson Inst. **12**:321–343. 1942.

RELATIONSHIP OF PHOTOPERIOD AND NITROGEN NUTRITION TO INITIATION OF FLOWER PRIMORDIA IN SOYBEAN VARIETIES

N. J. SCULLY,[1] M. W. PARKER,[2] AND H. A. BORTHWICK[3]

Introduction

Soybean varieties are known to differ in the degree to which they are controlled by photoperiod (2,7). Some varieties flower only when subjected to short photoperiods, while others flower even though they receive very long ones. A number of varieties have been reported as frequently showing significantly increased yield of forage and seed when they are grown on soils high in fertility (with respect to mineral elements) as compared with those which are low (4, 10). With other varieties the yield produced at a low level of fertility is asserted to be more frequently than not equal to that produced at a high level (5, 15). Such a lack of response to variations in soil fertility could perhaps result from the action of some factor of the plant or its environment that might be limiting. An unusual nutritional response in yield is occasionally reported when varieties that show the latter behavior and those that show the former are grown together under different levels of fertility. In this case the yield of one variety will significantly exceed that of another when they are grown at a high fertility level, while at a low level the relative yield of the two varieties is reversed (5, 15).

In previous studies of the influence of various levels of fertility upon yield of different varieties of soybean, no attention has been given to initiation of flower primordia. It is of interest to know to what extent the growth of such varieties is affected by photoperiod and to determine whether or not differences in time and place of initiation of flower primordia are in any way correlated with the yield differences observed when plants are supplied variable amounts of nitrogen. The present study considers the initiation of flower primordia and expression of other morphological characteristics for plants of four varieties of soybean. Two of the varieties are reported to show increased yields with increased level of fertility, while the yields of the other two are said to be unchanged by different degrees of fertility (4, 5, 9, 15).

Material and methods

Each experiment was concerned with only two of the four varieties of soybean, Morse and Virginia being used in some experiments and Lincoln and T-48 in others. The first two varieties have been in cultivation for many years and the last two were recently developed at the Illinois Agricultural Experiment Station, Urbana, Illinois.[4] The yields of Morse and Lincoln—but not those of Virginia and T-48—are reported to be influenced by fertility level (9, 15).

Plants were grown both in the greenhouse and in controlled environment rooms (13). In two preliminary experi-

[1] Associate Physiologist, [2] Physiologist, and [3] Senior Botanist; Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, Beltsville, Maryland.

[4] Seed of these varieties was supplied by E. J. KRAUS, University of Chicago, and by W. J. MORSE and J. L. CARTTER, Division of Forage Crops, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland.

ments, designed to study the photoperiodic requirements of these particular varieties, the plants were grown in soil in the greenhouse. In the other three experiments the plants were grown in sand cultures in either the greenhouse or control rooms, and with various lengths of photoperiod and various amounts of nitrogen added as sodium nitrate. The variations in sodium concentration were not considered of importance, since soybean is a nonaccumulator of this element (3).

Five nutrient solutions (table 1) were employed in the nutrition experiments. All were complete solutions and varied in amount of nitrogen. Solutions supplying a low, intermediate, and high level of nitrogen, respectively, were used in each experiment. Solution 4 (table 1), supplying the intermediate level of nitrogen at rate of 52 p.p.m., was previously developed for growing Biloxi soybean in sand culture, and an apparently normal growth of plants was obtained when it was employed in these experiments. Minor elements were supplied by a modified Hoagland solution (8). Solutions were applied daily in quantities sufficient to provide thorough flushing of the pots.

The plants grown in control rooms received light from a combined carbon-arc–Mazda source of approximately 1200 foot-candles at the surface of the pots (14). Those grown in the greenhouse received photoperiods both longer and shorter than those occurring naturally. In the former case supplemental light of approximately 50 foot-candles from 100-watt incandescent filament bulbs was employed to extend the natural photoperiod; and in the latter the plants were covered with double-thickness, black sateen cloth or were moved into dark chambers after they had received the required photoperiod.

Throughout the course of each experiment, the position of the first-flower node,[5] the number of nodes at which flower primordia were formed, the total nodes in the main axis, and the presence or absence of terminal inflorescences were determined for plants of the various treatments by microscopic observation. The *t*-test (17) was employed to measure the significance of differences occurring between plants of the various treatments. Differences significant at the 1% and 5% levels are reported as highly significant and significant, respectively.

TABLE 1

COMPOSITION OF NUTRIENT SOLUTIONS

Solution no.	Molar concentration of C.P. salts				N (p.p.m.)
	$MgSO_4 \cdot 7H_2O$	$CaCl_2 \cdot 2H_2O$	KH_2PO_4	$NaNO_3$	
1	0.0058	0.0030	0.0015	0.0010	14
2	.0058	.0030	.0015	.0015	20
3	.0058	.0030	.0015	.0018	26
4	.0058	.0030	.0015	.0037	52
5	0.0058	0.0030	0.0015	0.0075	105

Experimentation and results

Experiment I

An initial experiment was carried out to determine the effect of various lengths of photoperiod on the formation of flower primordia in the varieties Lincoln and T-48. On March 5, 1943, a planting of seed of each variety was made in the greenhouse in soil in 4-inch pots, 144 pots for a variety. The plants were sub-

[5] By "first-flower node" is meant that node on the primary axis of the plant at which the first flower primordia are formed. The cotyledons are located at node one, the primary leaves at node two, and the first compound leaf at node three.

jected to 20-hour photoperiods from time of germination until March 19. Ten plants of each variety were dissected on the latter date and no flower primordia were present on any plant. Beginning at this time, twenty plants of each variety were subjected to daily photoperiods of 12, 14, 16, and 20 hours, respectively.

On March 27, eight plants of each variety were dissected, and flower primordia were present on plants of both varieties on the 12- and 14-hour photoperiods, while none were present on those of the 16- and 20-hour lots. The remaining twelve plants of each variety on 12-, 14-, 16-, and 20-hour photoperiods were dissected on April 21, when the experiment was terminated. Although flower primordia were present on all plants of the 12-, 14-, and 16-hour lots, and on some plants of the 20-hour lots (table 2), nevertheless there were differences between varieties and between photoperiods in the position of the first-flower primordia formed. With the 20-hour photoperiod, however, flower primordia were present on only two of the twelve T-48 plants, while they were present on all twelve of the Lincoln plants. The average position of the first-flower primordia on plants of each of the three shorter photoperiods, respectively, was approximately one node lower for Lincoln than it was for the T-48; with the 20-hour photoperiod this difference between varieties did not occur. Lincoln plants did not form a terminal inflorescence with any of the four photoperiods and were still forming vegetative structures at the apex of the main stem, while T-48 plants formed such an inflorescence when grown on each of the three shorter photoperiods.

TABLE 2

EFFECT OF PHOTOPERIOD ON EXPRESSION OF CERTAIN MORPHOLOGICAL CHARACTERISTICS OF LINCOLN AND T-48 SOYBEAN VARIETIES GROWN IN SOIL IN THE GREENHOUSE. SEED PLANTED MARCH 5 AND DATA COLLECTED APRIL 21, 1943. TOTAL OF TWELVE PLANTS PER LOT

Variety	Photoperiod (hours)	No. plants flowering	Average node at which first-flower primordia occurred	No. plants with terminal inflorescence
Lincoln	12	12	5.0	0
	14	12	5.8	0
	16	12	6.5	0
	20	12	13.6	0
T-48	12	12	6.2	12
	14	12	7.2	12
	16	12	7.8	12
	20	2	14.0	0

EXPERIMENT II

On April 10, 1943, an experiment was started in the greenhouse with Lincoln and T-48 in which the plants were subjected to two different lengths of photoperiod and three different conditions of nitrogen nutrition. Eight treatment blocks were established, each consisting of six rows and ten pots per row. Three successive rows of each block were restricted to a variety. The relative order of varieties was alternated throughout the eight blocks.

Nutrient solutions 3, 4, and 5 (table 1), varying in amount of nitrogen, were employed, a different solution for each row of a variety within a treatment block. Solutions 3, 4, and 5 supplied nitrogen at rates of 25, 52, and 105 p.p.m., respectively, and were first applied immediately after planting of seed on April 10. All plants were maintained on 18-hour photoperiod until April 21, at which time the plants were thinned to one per pot and four of the treatment blocks were started on a 14-hour and the four others on an 18-hour

photoperiod. These treatments were continued until the experiment was terminated on June 15, 1943.

Throughout the experiment several dissections were made of plants from the various treatment lots. The first of these, made on April 21 when the plants were thinned to one per pot, consisted of ten plants of each variety from each of the three nitrogen levels. Each lot of ten plants had developed an average of eight nodes per plant, and no flower primordia were present on any plant. On April 29, three plants of each variety on both durations of photoperiod and the low and high nitrogen treatments were dissected. The plants were obtained from pots that served as guard rows for the various treatment blocks. On the 18-hour photoperiod the only plants with flower primordia were those of Lincoln grown with the low nitrogen treatment. With the 14-hour photoperiod, all plants except those of T-48 on the low nitrogen treatment had flower primordia. A third dissection, identical in size of sample with that of April 29, was made on May 6. Flower primordia were found on all plants except those of T-48 grown with an 18-hour photoperiod.

On May 22, plants from two of the 14-hour photoperiod blocks and two 18-hour blocks were harvested and dissected. The position of the first-flower node was determined for all plants of both photoperiods. The total number of nodes at which flower primordia were formed, and the total number of nodes in the main axis, were obtained only for the plants that had received 18-hour photoperiods (table 3). Similar data could not be obtained with accuracy for the plants on 14 hours because of the compactness of their terminals. The average position of the first-flower primordia for Lincoln and T-48 was at the 5.5 and 6.8 nodes, respectively, when plants were grown with the intermediate nitrogen treatments on the 14-hour photoperiod. An obviously significant difference between varieties in the location of first-flower primordia resulted when they were grown with this nitrogen treatment and an 18-hour photoperiod. In this case the primordia were developed at nodes 8.0 and 12.8 for plants of Lincoln and T-48, respectively. The same difference also existed for the high and low nitrogen treatments. Since no significant difference occurred among the various nitrogen treatments of either variety for position of the first-flower node when grown on the 14-hour photoperiod, the only data recorded in table 3 are for plants on the 18-hour photoperiod.

The position of the first-flower primordia on plants of T-48 on 18-hour photoperiod was influenced by the composition of the nutrient solution. In this case the difference between lots of the high as compared with those of the intermediate and low nitrogen treatments was highly significant. The greatest difference in position of first-flower node for plants of Lincoln occurred between those grown on the low and those on the high nitrogen treatments, but this difference was not statistically significant.

Highly significant differences in the number of nodes in the main axis that bore flower primordia occurred for plants of T-48 grown with the highest and intermediate nitrogen concentrations as compared with those at the low nitrogen concentration. No significant differences occurred in number of nodes bearing flower primordia for plants of Lincoln on the three nitrogen concentrations. On the other hand, while T-48 plants developed a decreasing number of flower nodes with increasing concentration of nitrogen, Lincoln plants developed a

greater number of flower nodes with the intermediate and high concentrations as compared with the low.

On the 18-hour photoperiods only Lincoln plants displayed a highly significant difference in total nodes. Such difference occurred between plants grown with low as compared with either those at intermediate or those at high nitrogen concentration. In general, with both varieties an increasing number of nodes were developed with increasing amounts of available nitrogen.

factory morphological data could not be obtained by the microscopic methods employed.

With the 18-hour photoperiod all the T-48 plants at each of the three concentrations of nitrogen had initiated a terminal inflorescence. Based on a total of twenty plants, the average node position above which terminal inflorescences were formed was 20.7, 23.4, and 24.1 for plants grown with the low, intermediate, and high concentrations, respectively. The Lincoln plants were just beginning to

TABLE 3

EFFECT OF NITROGEN CONCENTRATION ON EXPRESSION OF CERTAIN MORPHOLOGICAL CHARACTERISTICS OF LINCOLN AND T-48 SOYBEAN VARIETIES GROWN WITH SAND CULTURE IN THE GREENHOUSE ON 18-HOUR PHOTOPERIOD. SEED PLANTED APRIL 10 AND DATA COLLECTED MAY 22, 1943. AVERAGE OF TWENTY PLANTS PER LOT

Variety	Nitrogen concentration in nutrient (p.p.m.)	Average node at which first-flower primordia occurred	Total flower-bearing nodes	Total nodes
Lincoln	26	7.2±1.3	8.9±0.9	18.9±0.7*
	52	8.0±1.3	11.0±1.6	22.1±0.7
	105	9.2±1.1	10.0±1.6	22.4±0.7
T-48	26	12.0±0.3	5.0±0.5	19.4±0.5
	52	12.8±0.4	4.9±0.7	21.0±0.4
	105	15.6±0.8*	2.5±0.5*	21.5±0.8

* Significantly different from other two treatments at 1% level.

The sixty plants of each of the two remaining 18-hour photoperiod blocks were harvested and dissected on June 15, when the experiment was terminated. The main objective of this dissection was to locate the position of the terminal inflorescence for plants of each variety, but it also afforded an opportunity to recheck the position of first-flower primordia as determined by the dissection of May 22. The plants of the remaining 14-hour photoperiod blocks were not dissected, since as early as May 22 the nodes and internodes in the terminals had become so compacted that satisform terminal inflorescences. Of a total of twenty plants, one Lincoln plant formed a terminal inflorescence at the low nitrogen level, while eight and nine plants from lots of twenty each had done so at the intermediate and high concentrations, respectively. These inflorescences were formed above the 23.0, 25.5, and 25.7 nodes for plants of the low, intermediate, and high concentrations, respectively.

As was expected, the locations of the first-flower primordia for plants of Lincoln and T-48 were the same as observed on May 22. These primordia were de-

veloped on Lincoln at the 7.2, 8.2, and 9.2 nodes, respectively, for plants grown with the low, intermediate, and high concentrations, while on T-48 plants the first-flower node was at the 11.9, 12.7, and 15.5 node, respectively, with these nitrogen concentrations.

Experiment III

Plants for this experiment were grown with sand culture in 2-gallon glazed crocks with bottom drains and in a controlled-environment room maintained at 70° ± 1° F. Seed of Lincoln and T-48 varieties was planted August 18, 1943. The twenty-four crocks of a variety occupied half of each of two opposite, semicircular benches. The blocks of the respective varieties alternated from one bench to the other. The benches were so constructed and oriented with reference to the light source that equal light energies fell at the surface of all pots.

Solutions 2, 4, and 5 (table 1), supplying nitrogen at 20, 52, and 105 p.p.m., respectively, were applied to the plants, a different solution for eight crocks of each variety. The plants were subjected to an 18-hour photoperiod, beginning with germination of seed. On August 23 the plants were thinned to three per crock, and three plants of a variety on each of the three nitrogen treatments were dissected. The plants had no flower primordia and each had a total of eight nodes.

The 18-hour photoperiod and the three nitrogen treatments were continued until September 25, at which time the experiment was terminated and all plants were dissected. The node at which the first-flower primordia occurred, the number of nodes bearing recognizable flower primordia, and the total number of nodes in the main axis were observed for each plant. The data are shown in table 4.

TABLE 4

Effect of nitrogen concentration on expression of certain morphological characteristics of Lincoln and T-48 soybean varieties grown in controlled-environment room with sand culture and 18-hour photoperiods. Seed planted August 18 and data collected September 25, 1943. Average of twenty-four plants per lot

Variety	Nitrogen concentration in nutrient (p.p.m.)	Average node at which first-flower primordia occurred	Total flower-bearing nodes	Total nodes
Lincoln	20	9.5±0.7†	3.5±1.1	16.5±1.5*
	52	11.5±0.8	5.9±1.6	21.3±1.1
	105	11.8±0.9	6.0±1.4	21.7±0.9
T-48	20	10.8±0.5†	4.2±1.1	17.9±1.3
	52	12.8±0.6	3.2±1.0	19.7±1.0
	105	13.1±0.8	3.6±1.1	20.1±0.8

* Significantly different from other two treatments at 1% level.

† For T-48, significantly different from other two treatments at 5% level; for Lincoln, significantly different from 105 p.p.m. nitrogen treatment at 5% level.

In general, the data indicate trends similar to those observed for plants grown in the greenhouse where comparable photoperiod and nitrogen treatments were employed (experiment II; table 3). Differences significant at the

5% level were observed for node position of first-flower primordia with Lincoln plants grown at the low and the high nitrogen levels. There was no significant difference between plants of any other two treatments. Differences significant at the 5% level occurred for position of first-flower primordia of T-48 plants grown at low in comparison with either intermediate or high nitrogen levels.

With respect to total flower nodes there were no significant differences between plants of either Lincoln or T-48 at the various nitrogen levels. However, as was the case in experiment II, plants of the Lincoln variety had an increasing number of flower nodes with increasing nitrogen concentration, while with T-48 plants the highest number of flower nodes was developed at the lowest nitrogen concentration.

With increasing concentration of nitrogen there was an increasing number of nodes developed on the main axis for both Lincoln and T-48 plants. The difference in number of nodes was highly significant for plants of Lincoln grown with low as compared with either the intermediate or high nitrogen levels. In experiment II (table 3) this same degree of difference was observed only between plants of the low and high nitrogen levels. The difference in total nodes of T-48 plants grown with the various levels of nitrogen was not significant, however. This parallels the results observed for plants of this variety grown in the greenhouse experiment (table 3).

Experiment IV

In this experiment the effects of duration of photoperiod on the initiation of flower primordia and the differentiation of terminal inflorescences were observed for Morse and Virginia varieties. On June 21, 1943, plantings of each variety were made in soil in 4-inch pots in the greenhouse. Plants were thinned to one to a pot, and ten of each variety were subjected to photoperiods of 8, 10, 12, 14, 16, and 18 hours.

Two plants of each variety on each duration of photoperiod were dissected on July 10. Flower primordia were present on all those grown with 8-, 10-, and 12-hour photoperiods. All Virginia plants had developed terminal inflorescences on these photoperiods but the Morse plants had not. Flower primordia were also present on the Virginia plants of the 14-hour photoperiod but terminal inflorescences were not developed. One of the Morse plants on 14-hour photoperiod had developed flower primordia and none of either variety on 16- or 18-hour photoperiod had formed them.

The remaining eight plants of each variety on the various photoperiods were dissected when the experiment was terminated on July 22, and the data are shown in table 5. By this time both varieties had initiated flower primordia at all photoperiods from 8 to 18 hours inclusive, but, as was the case for Lincoln and T-48, the development of first-flower primordia was progressively delayed in both varieties by the longer photoperiods (table 5). Plants of Virginia formed terminal inflorescences when grown on photoperiods up to 14 hours, inclusive. No such inflorescence was developed by any Morse plant when grown with these photoperiods nor by plants of either variety when grown with longer photoperiods. On 8- to 16-hour photoperiods Virginia initiated first-flower primordia at a lower node position than did Morse. This means that flower-bud formation occurred earlier on Virginia than on Morse when the two varieties were grown with short photoperiods. On 18 hours, however, there was little dif-

ference in relative earliness, the node position of first-flower primordia being about the same for the two varieties. Thus varieties may differ in relative earliness when grown with long and short photoperiods.

TABLE 5

EFFECT OF PHOTOPERIOD ON EXPRESSION OF CERTAIN MORPHOLOGICAL CHARACTERISTICS OF MORSE AND VIRGINIA SOYBEAN VARIETIES GROWN IN SOIL IN THE GREENHOUSE. SEED PLANTED JUNE 21 AND DATA COLLECTED JULY 22, 1943. TOTAL OF EIGHT PLANTS PER LOT

Photoperiod (hours)	No. plants with flower primordia		Average node at which first-flower primordia occurred		No. plants with terminal inflorescence	
	Morse	Virginia	Morse	Virginia	Morse	Virginia
8	8	8	5.0	3.0	0	8
10	8	8	5.7	3.4	0	8
12	8	8	5.6	3.6	0	8
14	8	8	8.5	5.6	0	8
16	8	8	9.9	8.4	0	0
18	8	8	14.0	14.5	0	0

EXPERIMENT V

Plants of this experiment were grown in sand cultures in a controlled-environment room and with methods similar to those used in experiment III. Seed of Morse and Virginia were planted on July 15, 1943. Solutions 1, 4, and 5 (table 1) were employed which supplied nitrogen at rates of 14, 52, and 105 p.p.m., respectively. Eight crocks of each variety received the same solution. The plants were subjected to a 15-hour photoperiod immediately following germination, and were thinned to four to a crock on July 24. At this time they were expanding their first compound leaf. Two plants of a variety at each nitrogen level were dissected at this time and no flower primordia were present. All Morse plants had developed eight nodes, while those of Virginia had 7.6 nodes with the low and intermediate nitrogen levels and 8.0 with the high level. The plants of each variety on each nitrogen level removed at the time of thinning were subjected to chemical analysis. The samples for analysis consisted of the portion of the plant above the primary leaves. Ammonia, nitrate, and soluble nonprotein nitrogen were determined according to methods reported earlier (13). There was no appreciable difference in the ammonia content, and the values are not reported. The data (table 6) indicate that plants of each variety grown with different concentrations of nitrogen contain different percentages of nitrate and soluble nonprotein nitrogen. With both varieties the percentages of nitrate nitrogen for plants with intermediate concentrations were more than double those for plants grown with low nitrogen concentration. The percentage of nitrate nitrogen in plants supplied intermediate and high nitrogen concentrations were more nearly alike. The percentage of

TABLE 6

PERCENTAGE OF NITRATE AND SOLUBLE NONPROTEIN NITROGEN IN MORSE AND VIRGINIA SOYBEAN VARIETIES GROWN WITH SAND CULTURE FOR 9 DAYS IN CONTROLLED-ENVIRONMENT ROOM. PLANTS SUBJECTED TO 15-HOUR PHOTOPERIOD AND VARIOUS CONCENTRATIONS OF NITROGEN. DATA BASED ON FRESH WEIGHT OF TISSUE

Variety	Nitrogen in nutrient solution (p.p.m.)	Nitrate nitrogen (%)	Soluble nonprotein nitrogen (%)
Morse	14	0.021	0.253
	52	.044	.272
	105	.046	.310
Virginia	14	.022	.166
	52	.052	.210
	105	0.057	0.234

soluble nonprotein nitrogen of plants increased with increasing amounts of nitrogen in the nutrient solution, but the magnitude of the difference from the low to the high concentration was considerably less than that which occurred for the percentage of nitrate nitrogen. The chemical analysis data indicate that the nitrate and soluble nonprotein nitrogen content of the seedlings reflect the nitrogen content of the nutrient solution after only 9 days of differential nitrogen treatment.

The plants were grown with the 15-hour photoperiod and the various nitrogen treatments until August 12, at which time the experiment was terminated and all plants dissected. The node at which the first-flower primordia occurred, the number of recognizable flower-bearing nodes, and the total nodes in the main axis were observed for each plant. The data are given in table 7.

The various nitrogen treatments did not result in significant differences in position of the node bearing the first-flower primordia in either variety. The first-flower primordia of Morse occurred at nodes 8.9 and 9.9 on plants receiving 14 and 105 p.p.m. of nitrogen, respectively. Virginia plants formed their first-flower primordia at 8.1 and 7.8 nodes, respectively, with these nitrogen concentrations.

With respect to total flower-bearing nodes, there was an increasing number formed on plants of both varieties with increasing nitrogen concentration. No significant differences for total flower nodes occurred for plants of Morse, while highly significant differences occurred for those of Virginia grown on the low in comparison with the intermediate or high nitrogen levels. The difference in total nodes of Morse plants grown with the low nitrogen level and those with the high was highly significant. The difference between the total nodes of the plants grown with low and intermediate nitrogen levels was significant at the 5% level. With Virginia significant differences occurred between plants grown with the low and either the intermediate or the high nitrogen level.

TABLE 7

EFFECT OF NITROGEN CONCENTRATION ON EXPRESSION OF CERTAIN MORPHOLOGICAL CHARACTERISTICS OF MORSE AND VIRGINIA SOYBEAN VARIETIES GROWN IN CONTROLLED-ENVIRONMENT ROOM WITH SAND CULTURE AND 15-HOUR PHOTOPERIOD. SEED PLANTED JULY 15 AND DATA COLLECTED AUGUST 12, 1943. AVERAGE OF THIRTY-TWO PLANTS PER LOT

Variety	Nitrogen concentration in nutrient (p.p.m.)	Average node at which first-flower primordia occurred	Total flower-bearing nodes	Total nodes
Morse	14	8.9±0.5	2.3±0.7	14.8±0.7
	52	9.7±0.5	3.9±0.8	17.5±0.8†
	105	9.9±0.3	3.9±0.7	17.6±0.7*
Virginia	14	8.1±0.3	2.4±1.0	14.4±0.8
	52	8.0±0.6	5.5±0.9*	17.2±0.7†
	105	7.8±0.4	5.6±1.0*	17.3±0.8†

* Significantly different from 14 p.p.m. nitrogen treatment at 1% level.
† Significantly different from 14 p.p.m. nitrogen treatment at 5% level.

Discussion

BORTHWICK and PARKER (2) report that certain soybean varieties initiate flower primordia only when subjected to short photoperiods, while others initiate such primordia even though the plants receive continuous illumination. With varieties showing the latter response the effect of photoperiod manifests itself through a shift in the position at which the first-flower primordia are formed on the plant. The varieties Morse, Virginia, Lincoln, and T-48 are capable of initiating flower primordia on relatively long photoperiods and may possibly be able to do so with continuous illumination. The first-flower primordia of the varieties were initiated at a slightly higher node position on the plant axis with photoperiods of increasing duration up to 14 hours. With photoperiods of 16 hours, flower primordia were produced at positions considerably higher for each variety than they were with photoperiods of 14 hours, and with further increases in photoperiod these differences became greater. This is interpreted to mean that with daylengths of 16 hours and longer the factor of photoperiod becomes more critical for floral initiation and that slight changes in duration of photoperiod at this level result in marked differences in floral initiation. Although such varieties as Morse, Virginia, Lincoln, T-48, and others (2) can initiate flowers on extremely long photoperiods, the fact that they initiate them at progressively lower node positions with decreasing daylength substantiates the view that these varieties should be regarded as short-day plants.

The data in the literature on the nutrient requirements of soybean for optimum plant development are somewhat conflicting. This appears to be due in part to a factor of varietal adaptability which commonly occurs when varieties are grown on soils of different fertility or in localities in which other factors of the environment may likewise be limiting (11). The more recent field work on soybean nutrition has been carried out in the Midwest cornbelt area. LANG (10), in summarizing recent fertility studies, states that, in general, soybean varieties are adapted to a wide range of soil types and yield better on comparatively infertile acid soils than do any other crop plants in the cornbelt area. However, it is stated that for heavy yields they generally require relatively large quantities of plant nutrients, in comparison with other crop plants such as corn, and show a marked yield response to variations in natural soil productivity and to those treatments that develop soils to a favorable fertility for other crop plants which may follow (10). Based on field experiments, NORMAN (12) reports that soybean can effectively use more nitrogen than is provided through nitrogen fixation in the plant. The results of these workers suggest that some varieties may respond to different levels of fertility while others do not, when they are grown on certain natural daylengths.

POEHLMAN (15) reported that Morse variety in the field outyielded Virginia on soils of high fertility. On the less fertile soils the difference in forage yield between the two varieties diminished, and on the least fertile soils Virginia outyielded Morse. This reversal in variety yield with change in fertility level has also been noted by ETHRIDGE and HELM (5). MORSE (11) likewise reports that Virginia is adapted to soils of low fertility but makes no comparison of its behavior with the Morse variety. In experiments in which plants were grown with sand culture, ALLEN (1) observed that Morse was far superior to Virginia in forage

yields when both were grown with high concentrations of either potassium or magnesium. ETHRIDGE and HELM (5) found Virginia and Wilson varieties superior to Morse and Mikado in yield of both seed and hay when they were grown on Lintonia fine sandy loam soil which was low in fertility. In contrast to Virginia and Wilson, the growth of Morse and Mikado was short and stemmy and their seeds were borne so near the ground that considerable loss occurred in harvesting. Lincoln and T-48 have only recently been grown in the Midwest, and it is reported that the former responds to fertilizer while the latter does not (9).

The present study has considered the response of Lincoln, T-48, Morse, and Virginia to different levels of nitrogen only in terms of the expression of certain morphological characteristics. For this reason no attempt is made to correlate the present experimental results with the yield results of varieties under field conditions. However, certain data have been collected which may furnish a basis for further study of the problem.

The data indicate that although none of the four varieties investigated is prevented from flowering, the initiation of flower primordia in each is delayed by longer photoperiods. As noted earlier, when the factor of photoperiod alone was varied, the greatest differences in position of first-flower primordia occurred when the photoperiods were relatively long. When the factors of photoperiod and nitrogen were both varied in the initial experiments with Lincoln and T-48, the greatest changes in position of first-flower primordia again occurred with the longer daylengths. Similarly, significant differences for both the total nodes and the total number of nodes bearing flower primordia on the main axis occurred only when plants were grown with long photoperiods. A somewhat similar plant response to the factors of nitrogen nutrition and photoperiod has been noted in onion (16). Bulbing normally takes place only under long photoperiods, but when certain varieties are maintained on photoperiods at or near that critical for bulb formation the plants bulb earlier with low nitrogen concentration than with high. If the plants were to be grown for a sufficiently long period with these daylengths, however, those on low nitrogen would most likely form smaller bulbs. On the other hand, on photoperiods substantially longer than that critical for bulb formation there was no difference in bulb development when the plants were supplied different amounts of nitrogen.

The node position at which first-flower primordia of the soybean varieties were formed varied more in response to nitrogen nutrition when plants received long photoperiods than when they received short ones. The flower-forming stimulus of short photoperiods was so strong that flower primordia were formed when the seedlings were still very small. Floral initiation in these lots occurred before the influence of added nitrogen had become effective, so it is not surprising that variations in nitrogen nutrition were without effect on the position of first-flower primordia on such plants.

With long photoperiods, significant differences in position of first-flower primordia resulting from variations in nitrogen nutrition occurred in Lincoln and T-48 but not in Morse and Virginia. Morse and Virginia received a 15-hour photoperiod instead of an 18-hour one, however, and this may account for the difference in response of the two groups. The formation of first-flower primordia by both Lincoln and T-48 plants was

latest in the lots that received the greatest amount of nitrogen, and although significant differences occurred with both varieties, the differences were greater in T-48 than in Lincoln.

With increasing amounts of nitrogen, Morse and Virginia showed an increasing number of nodes bearing flower primordia. The same trend was shown for Lincoln, but the differences were not significant. On the other hand, T-48 showed the reverse trend. With this variety the effectiveness of the flower-inducing stimulus was evidently partially suppressed in plants at the higher nitrogen level because these plants had produced as many nodes and therefore as many positions for the formation of flower primordia as plants receiving the lower concentration of nitrogen. This result may be correlated in some degree with the fact that this variety is reported to respond less to field applications of fertilizer.

Two general types of development are recognized in soybeans. One is called determinate and the other indeterminate (**18**). The distinction is mostly concerned with the kind and location of the inflorescence. Development is indeterminate when no terminal inflorescence is formed and determinate when such an inflorescence is formed. The pod-bearing habit is also determined by the development habit of the plant and whether or not terminal inflorescences are formed.

On the basis of mode of pod formation, the determinate and indeterminate types have been described by ETHRIDGE, HELM, and KING (**6**). With the latter there is "a dense array of pods on the central stem, terminating there in a blunt apex, with a thin dispersal on the lateral branches; and, with the former, a sparse and comparatively even distribution of pods over all branches and stems, a diminishing frequency toward the tip of the central stem being notable." Whether a variety has a determinate or indeterminate type of development may be correlated with some of the types of morphological response obtained with Morse, Virginia, Lincoln, and T-48. At least on the basis of whether a terminal inflorescence is or is not formed under average field conditions, Morse and Lincoln are indeterminate and Virginia and T-48 are determinate types. The varieties Morse and Lincoln showed highly significant differences in total nodes with variation of nitrogen concentration on long photoperiods. Morse did not initiate terminal inflorescences on any photoperiods. Lincoln likewise failed to form terminal inflorescences, except for a few plants in experiment II in which they were just beginning to form at the time of harvest. On the other hand, Virginia and T-48, which displayed substantially less variation in total nodes with variation in nitrogen concentration on long photoperiods, initiated terminal inflorescences on all photoperiods up to 14 or 16 hours. It is of further interest that Virginia and T-48 are types which can show significantly less variation of yield due to variations in soil fertility than do Morse and Lincoln.

The type of data obtained from these experiments may have some bearing on the choice of varieties for late planting. They suggest that, if it were necessary to plant at such a time that the seedlings would immediately be subjected to short photoperiods, Morse might be more desirable than Virginia. Under such condition Virginia not only would begin to initiate flowers almost as soon as the plants came up but the plant would also form terminal inflorescences, thus limiting the number of places where

flowers and fruits could be borne. On the other hand, Morse plants would be expected to form first-flower buds slightly later than Virginia and to continue the differentiation of nodes and internodes at their terminals for a longer time, with the result that there would be more places for flower and fruit development.

Soybeans planted in the field during the late spring make their early growth at a time when photoperiods are longest and therefore least conducive to flowering. The data of this paper show that it is under these photoperiodic conditions that the plants of certain varieties exhibit the greatest differences in their response to variations in nitrogen treatment. These differences have been measured only in terms of variations in time and place of formation of flower primordia and in certain other morphological characteristics, and it is not known to what extent they may be responsible for ultimate differences in yield. It is significant, however, that rather great differences occur with respect to these plant characteristics in response to nitrogen treatment and that varieties differ rather markedly in the extent to which they exhibit such responses.

Summary

1. In a study of the effects of photoperiod and nitrogen nutrition on the expression of certain morphological characteristics of soybean, plants of Morse, Virginia, Lincoln, and T-48 varieties were grown either in controlled-environment rooms or in the greenhouse and in either soil or sand cultures.

2. In experiments in which nitrogen nutrition was not a factor, all varieties flowered on all durations of photoperiod, but first-flower primordia were formed at a higher node on the plant axis with the longer photoperiods.

3. Position of first-flower primordia did not vary with nitrogen treatment when the plants were grown with short photoperiods. When grown with long photoperiods, however, plants of certain varieties initiated first-flower primordia at higher nodes as the amount of nitrogen in the nutrient solution was increased. Variety T-48 was outstanding in this respect, Lincoln was intermediate, while Morse and Virginia were least responsive.

4. Plants of Lincoln and Morse grown on long photoperiods showed no effect of nitrogen concentration on the number of nodes bearing flower primordia, while there was an effect with Virginia and T-48. The number of flower nodes increased with increasing nitrogen concentration in the case of Virginia, while the opposite trend was true for T-48.

5. The total number of nodes per plant increased with increasing nitrogen concentration when Lincoln, Morse, and Virginia were grown on photoperiods that delay flowering, while this was not true for plants of the T-48 variety.

Bureau of Plant Industry, Soils and Agricultural Engineering
Beltsville, Maryland

LITERATURE CITED

1. Allen, Denver I., Differential growth response of certain varieties of soybeans to varied mineral nutrient conditions. Mo. Agr. Exp. Sta. Res. Bull. 361. 1943.
2. Borthwick, H. A., and Parker, M. W., Photoperiodic responses of several varieties of soybeans. Bot. Gaz. 101:341–365. 1939.
3. Bower, C. A., and Pierre, W. H., Potassium response of various crops on a high lime soil in relation to their contents of potassium, calcium, magnesium, and sodium. Amer. Soc. Agron. Jour. 36:608–614. 1944.
4. Carter, J. L., and Hopper, T. H., Influence of variety, environment, and fertility level on the

chemical composition of soybean seeds. U.S.D.A. Tech. Bull. 787. 1942.
5. ETHRIDGE, W. C., and HELM, C. A., Productive methods for soybeans in Missouri. Mo. Agr. Exp. Sta. Res. Bull. 195. 1922.
6. ETHRIDGE, W. C., HELM, C. A., and KING, B. M., A classification of soybeans. Mo. Agr. Exp. Sta. Res. Bull. 131. 1929.
7. GARNER, W. W., and ALLARD, H. A., Photoperiodic responses of soybeans in relation to temperature and other environmental factors. Jour. Agr. Res. 41:719–735. 1930.
8. HOAGLAND, D. R., and ARNON, D. I., The water-culture method for growing plants without soil. Univ. Cal. Agr. Exp. Sta., Berkeley, California. Circular 347. 1938.
9. KRAUS, E. J., Personal correspondence. 1943.
10. LANG, A. L., Facts on soybean fertilization. Soybean Digest 4:52–58. 1944.
11. MORSE, W. J., Soybeans now a major crop in the United States. U.S.D.A. Yearbook. 198–205. 1933.
12. NORMAN, A. G., Inoculation and nitrogen nutrition of soybeans. Soybean Digest 4:41–42. 1944.
13. PARKER, M. W., and BORTHWICK, H. A., Effect of photoperiod on development and metabolism of the Biloxi soybean. BOT. GAZ. 100:651–689. 1939.
14. PARKER, M. W., Environmental factors and their control. Soil Sci. (In press.)
15. POEHLMAN, J. M., A study of the relative adaptation of certain varieties of soybeans. Mo. Agr. Exp. Sta. Res. Bull. 255. 1937.
16. SCULLY, N. J., PARKER, M. W., and BORTHWICK, H. A., The interaction of nitrogen nutrition and photoperiod as expressed in bulbing and flower-stalk development of onion. BOT. GAZ. 106:52–61. 1945.
17. SNEDECOR, G. W., Statistical methods. Collegiate Press, Ames, Iowa. 1940.
18. WOODWORTH, C. M., Genetics and breeding in improvement of soybean. Univ. Ill. Agr. Exp. Sta. Bull. 384. Pp. 297–404. 1932.

THE ORGANIC ACIDS OF LEMON FRUITS[1]

WALTON B. SINCLAIR AND D. M. ENY

Introduction

The organic acids are the chief soluble constituents of lemon juice. As the fruit matures, the free acidity of the juice increases and the pH decreases. These changes are determined by estimating the organic acids by titrating a known volume of juice with standard NaOH, with phenolphthalein as indicator, and expressing the result as citric acid. The total acidity, as usually reported for lemon juice, represents the free acids determined in this way. Such values, however, do not show the relation between the kinds and amounts of organic acids in lemon fruits. The present study was carried out to determine these factors. The experiments involved the sampling of fruits of different sizes from a given plot of trees, at intervals during the growing season, and the subsequent determination, on the juice of each sample, of the soluble solids, the pH, the potentiometric titration curve, the free acids determined on both the juice and the lead acetate precipitate, the citric and malic acids, and the alkalinity of the ash. From these data, the relation of pH to the free and combined acids was determined.

In studies on the organic acids of orange juice, largely from mature fruits, SINCLAIR, BARTHOLOMEW, and RAMSEY (7) have shown that the total acidity of the juice is due chiefly to citric and malic acids, and that the variations in acidity are due chiefly to changes in the citric acid concentration (8.38–25.39 mg./ml. of juice). They found, also, a definite relation between the free-acid–combined-acid balance and the pH of the juice. The concentration of combined acids in the

[1] Paper no. 534, University of California Citrus Experiment Station, Riverside, California.

juice is remarkably uniform; this means that the free-acid concentration is the chief variable.

In other studies on Valencia oranges, SINCLAIR and RAMSEY (8) showed that the maximum amount of free acid was developed in the fruit early in the season and changed very little up to maturity. The concentration of free acids (milligrams per milliliter) in the juice, however, decreased considerably during fruit development. This decrease in free acidity with the corresponding increase in pH value was due chiefly to the decrease in concentration of citric acid brought about by an increase in volume of the fruit during the growing season. Although the malic acid concentration (milligrams per milliliter) in the juice stayed nearly uniform during the season, the actual amount in the fruit increased. The concentration of combined acid remained nearly uniform in the fruit, but the absolute amount per fruit increased. The amounts of combined acid determined from the alkalinity of the ash were in agreement with the values determined from the difference between the total- and free-acid radicals. During ripening, the changes in pH of the juice were definitely related to changes in percentages of the total-acid radical in the free form. A similar relation was noted between pH and the percentage of free acid expressed on a fresh-weight basis.

BARTHOLOMEW (1), in earlier studies, observed the decrease in pH value of lemon juice with the age of the fruit. Juice of Eureka lemons picked monthly between September and July showed a decrease in pH from 4.46 to 2.29. The greatest change in pH (4.46–2.91) occurred during the first 2 months of the sampling period and was accompanied by an increase in the water content of the fruit (53.97–75.42%). Although the titratable acidities are not recorded for these experiments, the large decrease in pH during the first half of the experimental period would necessitate a corresponding increase in the amount of free acid in the juice.

CALDWELL (2) has shown that, for a short period after the fruit has set, oranges and grapefruit have a pH value close to that found in the vegetative parts of the plant. During this period of low acid and high pH, the fruit has a high total-solids content. Subsequent to this period there is a short transition period (as compared with the total time required for fruit development), in which the pH rapidly decreases from the high values of the vegetative tissues to the low values of the growing fruit. This condition leads to an accelerated rate of water absorption, resulting in a reduction of total-solids content of the fruit and maximum hydration of the tissues.

Material and methods

The juice was extracted from the fruit samples (thirty to eighty lemons each, the number depending upon the size of the fruit), and a 300-ml. aliquot portion was centrifuged and used for the analyses. The refractive index was determined with an Abbé refractometer at 27° C., and the percentage of soluble solids and the specific gravity were read from a sucrose table. All pH values were determined with a Beckman glass-electrode pH meter at 23° C. For the titrations and the citric acid analyses, the juice was diluted with distilled water so that 25 ml. was equivalent to 1 ml. of juice.

The free-acid content of the juice samples was determined by two methods: (*a*) phenolphthalein titration and (*b*) potentiometric titration. The inflection point on the (potentiometric) titration curve

was taken to represent most accurately the amount of free acid in the juice.

The quantitative determination of citric acid, free and combined, was made on the juice samples by the pentabromacetone method of PUCHER *et al.* (**6**). The sample was heated with H_2SO_4 to convert the combined citrates to free citric acid, and the citric acid was oxidized to pentabromacetone by $KMnO_4$ in the presence of KBr. After extraction of the pentabromacetone with petroleum ether, the bromide ion was liberated with Na_2S and subsequently titrated with standard $AgNO_3$. The citric acid in the original sample was calculated from the titration. Since this procedure specifies a quantity of citric acid of 1 to 20 mg., it was necessary to modify the method slightly when juice samples containing more than 20 mg. citric acid per milliliter were being studied. The only change necessary was an increase in the amount of standard $AgNO_3$ added in the final steps in order to measure the bromide ion present. With lemon juice containing approximately 5% citric acid, an aliquot portion of diluted juice equivalent to 0.25 ml. pure juice was used for the pentabromacetone analysis.

The malic acid was precipitated from the juice by a procedure described by HARTMANN and HILLIG (**5**). The organic-acid fraction was precipitated from an alcoholic solution (80%) with lead acetate and the precipitate separated from the filtrate by centrifuging. The precipitate was washed twice with 80% alcohol, suspended in water, and freed of lead by passing H_2S through the solution. The lead sulphide was filtered off and washed with water. The filtrate and washings were combined and diluted to a known volume. The malic acid was determined on aliquot portions of this solution by the pentabromacetone method of PUCHER *et al.* (**6**). The principle of the method involved the oxidation of malic acid with $KMnO_4$, in the presence of KBr, to a bromine compound volatile with steam. This compound reacts with dinitrophenylhydrazine to give a water-insoluble product which is soluble in pyridine. A pyridine solution of this substance, when correctly diluted with water and made alkaline with NaOH, develops a blue color proportional to the amount of malic acid present.

The total acids (free and combined) were determined on the clear filtrate by the method of HARTMANN (**3**). An aliquot portion was evaporated to near dryness (one-tenth original volume), diluted (if necessary) to approximately 30 ml. with distilled water, and titrated with standard NaOH. The results were reported as citric acid. This value is equivalent to the total polybasic acids in the juice.

The amount of combined organic acid in the juice was found experimentally by measuring the alkalinity of the ash from an aliquot portion of juice. For each sampling, four 50-ml. aliquots of juice were ashed at 450° C. to a white residue. To this ash was added 5 ml. of standard 2N HCl; the whole was washed into a 100-ml. volumetric flask and diluted to volume with water; a 10-ml. aliquot was then titrated with standard NaOH. The difference in titer of NaOH between the sample and a blank solution of the HCl represents the alkalinity of the ash. The milliequivalents of HCl neutralized by the ash closely approximate the milliequivalents of combined organic acid in the juice, since these organic salts decompose to carbonates during ashing.

A plot of thirty lemon trees (fifteen Eureka and fifteen Lisbon) was the source of the fruit used in studying the relation of fruit size to the pH and free acidity of the juice. All fruits used in this

particular experiment were tagged and measured when 1.5–2.0 cm. in diameter. As lemon trees bloom and set fruit in each month of the year, it is necessary to know the size of the fruit at the beginning of the experiment in order to correlate the size with the age of the fruit. To secure the juice from small fruits 2–3 cm. in diameter, they were peeled carefully with a knife. The resulting pulp, which amounted to one-fourth to one-third the volume of the whole fruit, was placed in a jar and frozen solid in a cold-storage chamber. After thawing, the pulp was subjected to a pressure of 20,000 pounds per square inch in a hydraulic press. The expressed liquid was centrifuged and analyzed.

TABLE 1

RECOVERY OF MALIC ACID FROM PURE SOLUTIONS

MALIC ACID TAKEN (MG.)	MALIC ACID RECOVERED BY			
	Gravimetric method		Colorimetric method	
	mg.	%	mg.	%
1.00	0.99	99.0	1.00	100.0
1.00	1.06	106.0	0.95	95.0
1.00	0.99	99.0	1.03	103.0
2.00	1.99	99.5	2.08	104.0
2.00	2.06	103.0	2.03	101.5
2.00	1.99	99.5	2.00	100.0
3.00	3.06	102.0	2.98	99.3
3.00	2.99	99.6	2.98	99.3
3.00	2.92	97.3	2.90	96.6
5.00	5.06	101.2	5.20	104.0
10.00	10.25	102.5	9.90	99.0
Mean		100.8		100.2

Results

DETERMINATION OF MALIC ACID IN LEMON JUICE

The malic acid values determined by the colorimetric method represent to a considerable degree of accuracy (within 5%) the actual amount of malic acid in the juice samples. Nevertheless, it was thought desirable to compare the gravimetric and colorimetric methods on pure solutions of malic acid. The results obtained by both methods (table 1) gave satisfactory agreement.

In the gravimetric procedure, the factor reported by HARTMANN (4) was used to convert the hydrazine precipitate into malic acid (1 gm. hydrazine precipitate = 0.712 gm. malic acid). This factor is not significantly different from the experimental value determined in the present studies (1 gm. hydrazine precipitate = 0.715 gm. malic acid). The precipitates that were weighed in the gravimetric determinations were obtained by distilling the volatile bromine compound of malic acid into dinitrophenylhydrazine and were not the result of direct precipitation from the oxidized solution (4).

TABLE 2

RECOVERY OF MALIC ACID ADDED IN VARIOUS AMOUNTS TO LEMON JUICE*

EXPERIMENT NO.	MALIC ACID CONTENT (MG./ML.)	MALIC ACID ADDED (MG./ML.)	MALIC ACID RECOVERY FROM LEAD-ACETATE PRECIPITATE		
			Total (mg./ml.)	Added (mg./ml.)	%
1	2.30	0.00	2.30	0.00	.....
2	2.30	0.50	2.80	0.50	100.00
3	2.30	1.00	3.36	1.06	106.00
4	2.30	1.50	3.82	1.52	101.33
5	2.30	2.00	4.40	2.10	105.00

* Juice sample contained 63.90 mg./ml. citric acid.

The thoroughness with which lead acetate precipitates malic acid from lemon juice is indicated by the percentages of recovery listed in table 2. Different amounts of malic acid were added to aliquot portions of a lemon-juice sample and subsequently precipitated with lead acetate in 80% alcohol. The total amount of malic acid in this precipitate was determined as previously described. The lemon juice used for these determinations contained 63.90 mg. citric acid and 2.30

mg. malic acid per milliliter. The high concentration of citric acid served to test the effect of this organic acid on the recovery of malic acid.

Relation of free-acid fraction to citric acid content of lemon juice

In previous studies on orange juice (**7, 8**), the free acid calculated from the volume of standard NaOH required for neutralization, with phenolphthalein as indicator, was higher than the amounts determined by potentiometric titration. This was because the phenolphthalein changes color between pH 8 and 9, and more NaOH is required to reach the end point with phenolphthalein than to titrate the corresponding sample potentiometrically to pH 7.80—the inflection point on the titration curve. The titration curve of pure citric acid is nearly perpendicular to the abscissa in that portion of the curve close to the neutral point, but the titration curve of orange juice has considerable slope within a similar region of the curve.

With lemon juice, however, the free-acid values determined by the phenolphthalein and potentiometric titration methods gave good agreement (table 3). This was true for both green and mature lemons ranging from 4.0 to 7.0 cm. in diameter. The inflection point on the titration curve of lemon juice occurred at approximately pH 8.10, in the region where phenolphthalein gave an initial color change. In this region the curve of lemon juice, like that of pure citric acid, is perpendicular to the abscissa, and only a very small increment of NaOH is needed to produce a large change in pH.

The actual amount of citric acid in the lemon-juice samples, as determined by the pentabromacetone method (**6**), is shown in table 3. These values include both the free citric acid and that combined in the form of citrates. The total citric acid content is approximately equal to the free acid. It can be seen that additional organic-acid radicals are needed to account for the salt formation in the juice. These are supplied by the malic acid in the juice, as reported in table 5. The amount of organic acid in the salt form is reported as combined acid (table 3), calculated as citric acid from the alkalinity of the ash. The concentration of combined acid is comparatively uniform, when the wide range of maturity of the fruit samples is considered.

The citric acid content of various lemon-juice samples, determined on the

TABLE 3

RELATION OF FREE-ACID FRACTION TO CITRIC ACID CONTENT OF LEMON JUICE

Sample no. and variety	Sampling date	Stage of fruit maturity	Fruit diameter (cm.)	Total soluble solids (%)	pH	Free acid (as citric) determined by				Citric acid (pentabromacetone method)		Combined acids (as citric) determined from alkalinity of ash	
						Phenolphthalein titration		Potentiometric titration					
						mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.
1. Lisbon	11/30/44	Green	5.0	9.19	2.30	54.54	0.852	53.74	0.840	54.32	0.849	2.21	0.035
2. Eureka	12/ 6/44	Green	4.5	8.31	2.30	48.15	0.752	48.15	0.752	47.88	0.748	2.44	0.038
3. Eureka	12/12/44	Green	4.5	9.35	2.25	58.99	0.923	58.80	0.919	53.08	0.829	3.10	0.049
4. Lisbon	12/17/44	Silver	4.5	9.48	2.26	61.50	0.961	61.50	0.961	64.96	1.015	2.90	0.045
5. Lisbon	12/26/44	Silver	5.7	9.09	2.38	58.07	0.907	57.88	0.904	52.00	0.812	2.90	0.045
6. Eureka	1/10/45	Yellow	7.0	7.96	2.22	53.01	0.828	53.01	0.828	54.47	0.851	2.03	0.032
7. Eureka	1/16/45	Green	5.0	8.30	2.20	51.83	0.810	51.83	0.810	48.83	0.763	2.06	0.032
8. Lisbon	1/18/45	Yellow	5.0	9.01	2.18	56.68	0.885	56.68	0.885	56.45	0.882	1.60	0.025
9. Lisbon	1/22/45	Yellow	6.5	9.17	2.15	58.47	0.913	58.80	0.919	59.14	0.924	1.80	0.028
10. Lisbon	1/27/45	Yellow	5.5	8.54	2.22	55.15	0.862	55.48	0.867	54.10	0.845	2.06	0.032

pure juice and on the lead-acetate precipitate, is recorded in table 4. Attention should be drawn to the close agreement between the citric acid values determined in the two cases. Apparently, lead acetate gave a complete precipitation of the citric acid radical (free and combined) in the juice. The free acid (as citric) is not significantly different from the actual citric acid content of the pure juice. These data show further that the nonacid constituents in lemon juice do not influence the determination of citric acid by the pentabromacetone method.

TABLE 4

CITRIC ACID VALUES DETERMINED ON PURE LEMON JUICE AND ON LEAD-ACETATE PRECIPITATE

SAMPLE	FREE ACID (AS CITRIC) (MG./ML.)	CITRIC ACID DETERMINED ON	
		Pure juice (mg./ml.)	Lead-acetate precipitate (mg./ml.)
1	61.24	62.50	62.27
2	55.68	55.21	55.12
3	58.80	53.08	53.00
4	63.67	62.93	63.02
5	70.08	68.99	68.99
6	57.85	58.24	58.46
7	61.50	64.98	64.53
8	58.82	59.14	59.03
9	55.25	55.87	56.00
10	62.37	65.00	65.00

POLYBASIC ACIDS OF LEMON JUICE PRECIPITATED WITH LEAD ACETATE

According to HARTMANN (4), the organic polybasic acids occurring in fruits are soluble in alcohol and are precipitated quantitatively as the lead salts from 80% alcohol. Since the acidity of lemon juice is due chiefly to the polybasic acids, it follows that this procedure should serve to isolate them in nearly pure form from the other constituents in the juice. On this basis, the polybasic acids of lemon juice have been isolated and subsequently determined quantitatively by the oxidation methods. In table 5 the values are recorded as (1) total acid (as citric) titrated with standard NaOH, with phenolphthalein as indicator, (2) citric acid, (3) malic acid, and (4) total citric and malic acids. The first three values were experimentally determined.

The total-acid values (as citric) represent all the acid groups precipitated from the juice with lead acetate. This precipitate included, if present, the lead salts of the fruit acids, the combined acids, and the acid salts of the juice. These titration values are highly accurate, provided the lead-acetate precipitate is thoroughly washed with 80% alcohol to free it of excess lead acetate and acetic acid.

The polybasic acids precipitated from lemon juice with lead acetate are represented by the citric and malic acid values (table 5). It is obvious that the main portion of the total acids of lemon juice is citric acid. Although the concentration of malic acid is small in comparison with that of citric acid, the amount present has to be considered more than a trace, as usually reported in the literature. The malic acid concentration of lemon juice varied widely with the maturity of the fruit, however, the amount decreasing as the fruit ripened. The total-acid constituents (titratable acidity) of the lead-acetate precipitate are equal (within experimental error) to the sum of the citric and malic acids, expressed in milliequivalents per milliliter. This is important because it demonstrates that the actual amounts of citric and malic acids account for all the acid groups precipitated as the lead salts.

If isocitric acid is present in more than microconcentrations, it should appear in the lead-acetate precipitate. Isocitric acid does not yield pentabromacetone (4)

TABLE 5

ANALYSIS OF ORGANIC ACIDS OF LEMON JUICE

SAMPLE NO. AND VARIETY	SAMPLING DATE	STAGE OF FRUIT MATURITY	FRUIT DIAMETER (CM.)	TRUE* SOLUBLE SOLIDS (%)	pH	FREE ACID (AS CITRIC) DETERMINED BY PHENOLPHTHALEIN TITRATION		ACID CONSTITUENTS PRECIPITATED BY LEAD ACETATE								COMBINED ACID (AS CITRIC) DETERMINED FROM ALKALINITY OF ASH		FREE ACID (%)
								Total acid titrated (as citric)		Citric acid (pentabromacetone method)		Malic acid		Total acid (citric+malic)				
						mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	mg./ml.	me./ml.	
Fresh fruit from experimental plots																		
1. Lisbon	April 3	Yellow	6.0	8.8	2.20	55.25	0.863	59.02	0.922	56.00	0.875	3.65	0.055	59.54	0.930	1.76	0.028	96.90
2. Lisbon	April 9	Yellow	6.0	9.8	2.15	62.37	0.974	67.52	1.055	65.00	1.015	2.60	0.039	67.48	1.054	2.18	0.034	96.63
3. Lisbon	April 16	Green	4.0	9.7	2.13	55.94	0.874	61.72	0.964	58.52	0.914	2.60	0.039	61.01	0.953	1.88	0.029	96.74
4. Eureka	April 20	Yellow	5.0	9.0	2.25	55.94	0.874	61.21	0.956	59.77	0.934	1.50	0.022	61.20	0.956	1.94	0.030	96.64
5. Eureka	June 8	Green	4.5	8.0	2.33	45.56	0.712	49.67	0.776	46.59	0.728	2.96	0.044	49.42	0.772	1.83	0.029	96.15
6. Lisbon	June 12	Green	4.0	8.7	2.28	53.78	0.840	57.58	0.899	53.31	0.833	4.20	0.063	57.36	0.896	1.90	0.029	96.59
7. Eureka	June 18	Green	4.0	8.3	2.31	45.87	0.716	48.72	0.761	45.33	0.708	3.51	0.052	48.66	0.760	1.79	0.028	96.25
8. Lisbon	June 25	Green	4.0	8.8	2.28	48.09	0.751	53.12	0.830	47.87	0.748	4.28	0.064	51.98	0.812	1.86	0.029	96.27
Mature fruit from packing-house storage†																		
9. Eureka	April 23	Yellow	5.5	8.9	2.25	62.37	0.974	69.05	1.079	67.65	1.057	1.90	0.029	69.53	1.086	2.13	0.033	96.69
10. Eureka	April 25	Yellow	5.5	9.2	2.25	62.37	0.974	65.30	1.021	63.50	0.992	1.80	0.027	65.24	1.019	2.08	0.033	96.76
11. Eureka	April 30	Yellow	5.5	9.6	2.23	67.19	1.049	71.50	1.117	69.89	1.092	2.10	0.032	71.96	1.124	2.13	0.033	96.92
12. Eureka	May 2	Yellow	6.0	7.9	2.22	54.66	0.854	59.16	0.924	57.12	0.892	2.30	0.035	59.34	0.927	1.94	0.030	96.59
13. Eureka	May 7	Yellow	6.0	8.9	2.32	61.50	0.961	65.80	1.028	63.89	0.998	2.20	0.033	66.00	1.031	2.15	0.034	96.62
14. Eureka	May 10	Yellow	4.5	9.0	2.20	61.25	0.957	66.05	1.032	63.90	0.998	2.30	0.035	66.13	1.033	1.90	0.030	96.99
15. Eureka	May 14	Yellow	5.5	10.3	2.11	70.10	1.095	76.05	1.188	74.28	1.160	2.25	0.034	76.44	1.194	2.08	0.033	97.11

* Owing to the relatively high acid content of the juice, a correction was added to the refractometer sucrose values to give the true percentage of soluble solids (9).

† Samples taken from different lots of fruit that had been in storage from 1 week to 3 months.

and consequently has no influence on the citric acid values determined by the pentabromacetone method. The only possibility left would be for the isocitric acid to form another compound during the oxidation process and cause a slight increase in the malic acid values.

Relation of pH to free and combined acids in lemon juice

The free-acid fraction of lemon juice is due mostly to citric acid. The combined-acid fraction is calculated as citric. The error in this calculation is small, since the malic acid content is very low in comparison with the citric acid content, and the equivalent weights of the two acids are nearly equal (citric acid 64.02, malic acid 67.02). It is seen then that the buffering action of lemon juice is due to the weak acid (citric) and a salt, or salts (citrates), with a common anion. The pH of the juice is therefore determined chiefly by the dissociation constant (pK_a) and the ratio of combined acid to free acid. Other constituents that may contribute to the hydrogen-ion concentration in lemon juice are in such small amounts, in comparison with the citric and malic acid content, as to have little effect.

The relation of pH to the concentration of free acid (as citric, in milligrams per milliliter) in different samples of lemon juice is shown in table 5. The juice from fruits that ranged from 4.0 to 6.0 cm. in diameter showed only a slight variation in pH. Although the free acid (as citric) varied from 45.56 to 70.10 mg./ml., the percentage of total acid in the free state showed little variation. This means that the combined acids (salts of inorganic cations) did not vary enough to produce more than a slight shift in pH. The average amount of citric acid in the combined form was 1.97 mg./ml. This relation is further borne out by the titration curve of lemon juice shown in figure 1. These small fluctuations of pH correspond to changes in the salt content of 3.00–4.00%.

The relation of pH to the percentage of total acid in the combined form (salt) is well illustrated in figure 1 by the titration curves of lemon juice and of pure citric acid solutions. The concentration of the citric acid solution used in securing the data for one of the curves was 62.00 mg. citric acid per milliliter; the concentration of free acid in the lemon-juice sample was equivalent to 61.50 mg. citric acid per milliliter. Both the citric acid solution and the lemon-juice sample had, intentionally, nearly the same concentration of free acid. The initial pH value of this pure citric acid solution was 1.82; that of the lemon juice was 2.32. The initial point on the lemon-juice curve (fig. 1) was corrected for the salt occurring naturally in the juice (3.40% at pH 2.32). The percentage of acid neutralized, for all points other than the initial one, included the combined acids present in the original sample and the sodium citrate formed by the neutralization of part of the free acid. For purposes of this study, curves representing only 50% of the total acid neutralized have been plotted on the graph.

The slight difference between the curves (fig. 1) is indicated by the fact that the pH values on the lemon-juice curve are slightly higher than those on the citric acid curves, for a given amount of combined acid (salt). The curves are nearly parallel and consequently have nearly the same slope. The close similarity of these curves is evidence that lemon juice titrates like a pure citric acid solution, because the free acid in the juice is composed chiefly of citric acid, and the ratio of free acid/combined acid is sufficiently great to diminish the salt effect

on the pH. The quantity of cations available in the juice for combining with the organic acids is relatively small in comparison with the concentration of free acids. Nevertheless, to designate the pH-salt relationships accurately, a correction must be made for the combined acid naturally occurring in the juice. In titrating lemon juice with a strong base, it is possible to calculate the pH at any point on the curve preceding the end point, with the familiar equation pH = pK_a + log (salt/acid).

Since lemon juice titrates like a pure acid solution, its buffer value is equal to that of any other weak acid. All weak acids have dissociation curves of the same shape and have equal buffer capacities at any given degree of dissociation (or fraction of acid neutralized). Lemon juice, like any weak acid, has the greatest buffer efficiency when 50% of its acid is in the salt form. At this point the ratio salt/acid is unity, and the pH is equal to pK_a. At this point also the titration curve has a minimum slope and, consequently, a maximum buffer efficiency.

In previous papers on orange juice (**7, 8**) it was shown that the combined acids calculated as the difference between the free acid and the sum of the citric and malic acids were approximately the same as the combined acids determined from the alkalinity of the ash (all values calculated in milliequivalents). With lemon juice, however, the difference between the free-acid fraction and the total organic-acid radical (citric and malic) is relatively much greater than the combined acids determined from the alkalinity of the ash (table 5). Experimentally this means that a small portion of the acid radical was in a form other than free acid or salt of the inorganic cations, but that it was accounted for in the citric and malic acid fractions of the juice.

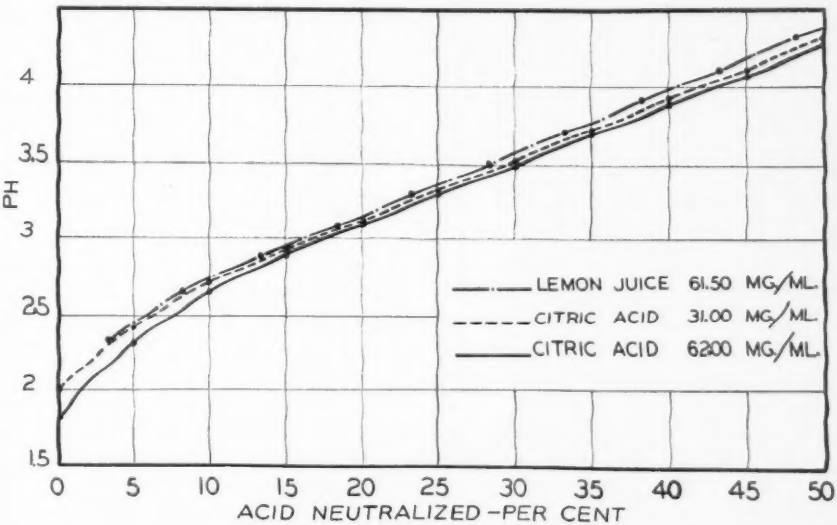


FIG. 1.—Titration curves of lemon juice and of pure citric acid solutions, showing relation of pH to salt content, up to 50% of acid neutralized. Initial point on lemon-juice curve was corrected for salt occurring naturally in the juice.

Since this particular form of the combined-acid radical was not titrated with NaOH, or did not exist in the juice as a salt of inorganic cations, the pH and titration curve were not affected on the acid side of the inflection point. With respect to factors that affected the pH, the alkalinity of the ash gave a true combined-acid content of the juice. This is well demonstrated in figure 1. The difference in the combined acids determined by the two methods strongly indicated that a small portion of the citric or malic acids existed in the ester form. This compound in the juice would fit the conditions just cited.

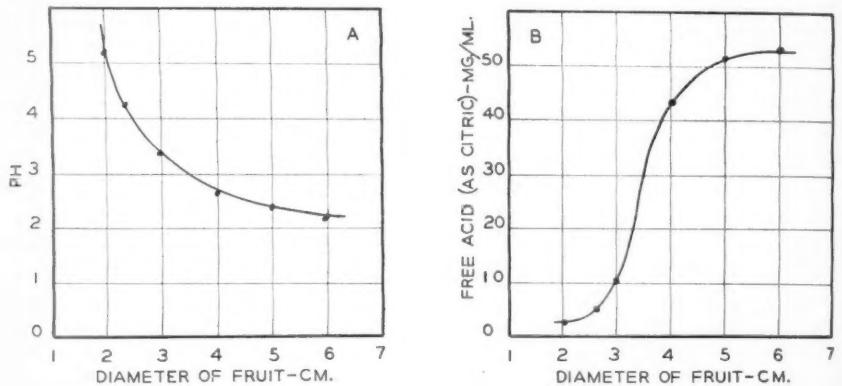


FIG. 2.—Changes in pH and in free acid with fruit growth. Each point on the curves represents the mean of many determinations. Fruits sampled had been previously tagged when 1.5–2.0 cm. in diameter.

CHANGES IN PH AND FREE ACID OF LEMON JUICE WITH FRUIT GROWTH

The pH and free-acid values of the juice of lemon fruits of different sizes are shown in figure 2. Results show the increase in free acids and decrease in pH with increase in fruit size. As the equatorial diameter of the fruit increased from 2.0 to 4.0 cm., the pH value of the juice decreased from 5.20 to 2.60. The large reduction in pH at this period of fruit growth is due to a correspondingly large increase in free acid. Further increase in fruit size, up to a diameter of 6.0 cm., resulted in a decrease of approximately 0.3 of a pH. After the free acid reached a certain concentration, which apparently appears when the fruit is about 4.0 cm. in diameter, the pH changed very little. The free acids, on the other hand, continued to increase gradually after the fruits had reached this diameter.

It should be emphasized that these fruits were picked according to size from the same trees throughout the sampling period. Each value on the two curves, *A* and *B* (fig. 2), is the mean of many determinations on fruits of the same bloom. These curves are trends and represent the change in pH and free acid in the juice of fruits of known ages. As lemon trees bloom and set fruit in each month of the year, the relationships shown in figure 2 would probably not hold for fruits picked at random at any one time. This is borne out by the data of table 5, which show that the juice of fruits 5.5 cm. in diameter may have more free acid (67.19 mg./ml.) than that of fruits 6.0 cm. in diameter (54.66 mg./ml.). To

overcome most of this difficulty, the fruits that were sampled had been previously tagged when they were 1.5–2.0 cm. in diameter. The size of the fruit may not indicate its age, unless fruits of the same bloom are used. BARTHOLOMEW (1), by tagging a great number of fruits and measuring at monthly intervals, showed the relation between the age and the diameters of lemon fruits. The curve (fig. 2) showing the change in pH with the size of fruit is in good agreement with BARTHOLOMEW's data showing the change in pH of lemon juice with the age of the fruit. It is evident, therefore, that if the pH and total acids, respectively, were plotted against the age of the fruit rather than against the size, the trend of the curves would be the same.

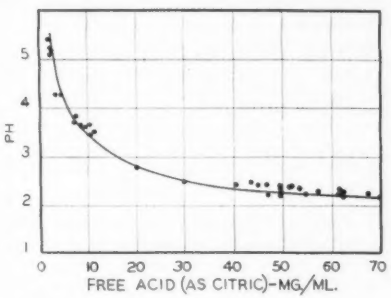


FIG. 3.—Changes in pH with variations in concentration of free acid in lemon juice during fruit development.

Figure 3 designates the changes in pH with variations in concentration of free acid (milligrams per milliliter) in fruits ranging between 1.5 and 6.0 cm. in diameter. This curve is interesting because it shows the rapid increase in the hydrogen-ion concentration of the fruit, from the low values approaching those of the vegetative tissues to the high values of the growing fruit. This curve demonstrates again that when the free acid reached a concentration of about 40 mg./ml., further increases produced only slight changes in pH.

Summary

1. The acidity of lemon juice is due chiefly to citric and malic acids. If other acids are present, they exist in very small amounts. The citric acid determined on the pure juice does not differ significantly from the amount precipitated by lead acetate. The total acid content precipitated from lemon juice by lead acetate is equal (within experimental error) to the sum of the citric and malic acids.

2. The difference between the free-acid fraction and the total organic-acid radical (citric and malic) is relatively much greater than the combined acids determined from the alkalinity of the ash. Since this difference is not reflected in the titratable acidity or pH values, it probably represents the quantity of organic acid in the ester form. The alkalinity of the ash represents the organic acid combined in salt form with the excess inorganic cations.

3. The titration curve of lemon juice is very similar to that of a pure citric acid solution, provided a correction is made for the combined acid naturally occurring in the juice. There is a definite relation between the pH and the amount of acid in the salt or combined form. The small fluctuations in pH of mature lemon juice are correlated with the large ratio of free acid/combined acid.

4. The free acids (milligrams per milliliter) increased and the pH of the juice decreased with increase in fruit size. The large reduction in pH (5.20 to 2.60) which occurred in fruits 2.0–4.0 cm. in diameter was due to a correspondingly large increase in free acid. Further increase in fruit size (up to 6.0 cm. in diameter) resulted in a slight decrease of approximately 0.3 of a pH, while the free

acids gradually continued to increase in the juice.

For the past 4 years, investigations on the organic acids of citrus fruits have been in progress at this station. During this period we have had at all times the benefit of discussions with the following members of the Research Department of the California Fruit Growers Exchange: Messrs. W. E. BAIER, J. W. STEVENS, and P. W. ROHRBAUGH; and Drs. GLENN H. JOSEPH and EDWIN F. BRYANT.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA

LITERATURE CITED

1. BARTHOLOMEW, E. T., Internal decline of lemons. II. Growth rate, water content, and acidity of lemons at different stages of maturity. Amer. Jour. Bot. **10**:117–126. 1923.
2. CALDWELL, JOSEPH S., Hydrion concentration changes in relation to growth and ripening in fruits. U.S. Dept. Agr. Tech. Bull. 403. 1934.
3. HARTMANN, B. G., Report on fruits and fruit products. Assoc. Off. Agr. Chem. Jour. **22**:355–362. 1939.
4. ———, The polybasic acids of fruits and fruit products. Assoc. Off. Agr. Chem. Jour. **26**:444–462. 1943.
5. HARTMANN, B. G., and HILLIG, F., Acid constituents of food products: Special reference to citric, malic, and tartaric acids. Assoc. Off. Agr. Chem. Jour. **17**:522–531. 1934.
6. PUCHER, G. W., VICKERY, H. B., and WAKEMAN, A. J., Determination of malic acid in plant tissue. Simultaneous determination of citric and malic acids. Ind. Eng. Chem., Anal. Ed. **6**:288–291. 1934.
7. SINCLAIR, W. B., BARTHOLOMEW, E. T., and RAMSEY, R. C., Analysis of the organic acids of orange juice. Plant Physiol. **20**:3–18. 1945.
8. SINCLAIR, W. B., and RAMSEY, R. C., Changes in the organic-acid content of Valencia oranges during development. BOT. GAZ. **106**:140–148. 1944.
9. STEVENS, J. W., and BAIER, W. E., Refractometric determination of soluble solids in citrus juices. Ind. Eng. Chem., Anal. Ed. **11**:447–449. 1939.

CARPELLARY AND PLACENTAL STRUCTURE IN THE SOLANACEAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 570

MARY AILEEN MURRAY

Introduction

There has been much speculation on the exact morphology of the carpel and placentae in the Solanaceae. The axile placentae and cauline ovules ascribed to this family by most workers are difficult to reconcile with the Candollean theory that all floral parts are specialized leaves. According to the classic theory, an ovary is composed of one or more carpels, each of which is morphologically comparable to a folded leaf bearing ovules on its margins. In a syncarpous ovary, however, the carpels are undiverged, and the axis may extend into the gynecium. The problem is to determine whether the carpel walls, the septa, and the ovule-bearing portions of the placentae are cauline, foliar, or both.

A total of fourteen genera and twenty-one species of this family were selected for investigation, including members with fleshy and with only slightly fleshy placentae. On the basis of the anatomy of each flower, it was noted that WETTSTEIN's (**23**) phylogenetic arrangement of the genera agrees rather closely with a sequence based on floral anatomy. While this paper is not specifically one on phylogeny, the phylogenetic approach proved the best method of co-ordinating the material.

The Solanaceae contain many plants of economic importance. These can be divided into three groups. *Solanum tuberosum* (potato), *S. nigrum* var. *guineense* (garden huckleberry), *S. melongena* var. *esculentum* (eggplant), and species of *Lycopersicon* (tomato) and *Capsicum* (pepper) are sources of food. *Nicandra physalodes* and species of *Lycium*, *Datura*, *Nicotiana*, *Petunia*, *Nierembergia*, *Salpiglossis*, *Schizanthus*, *Cestrum*, *Brunfelsia*, *Solanum*, and *Browallia* are cultivated as ornamentals. Several members are grown for the alkaloids they contain. These include *Atropa belladonna*, *Hyoscyamus niger*, *Datura stramonium*, and species of *Nicotiana*.

The flowers of the Solanaceae are hypogynous and generally actinomorphic, calyx four- to six-lobed and persistent, corolla sympetalous and generally five-lobed, stamens five, and the filaments adnate to the corolla and alternate with the corolla lobes. The ovary is usually bicarpellate, but in some plants there may be three to five carpels. The ovules are generally numerous and borne on more or less fleshy placentae. The fruit is a capsule or a berry. The Solanaceae are most closely related to the Scrophulariaceae but are separated from this family by having bicollateral bundles and an ovary which is oblique in relation to the median plane of the flower.

Among families in which the ovary is superior and bicarpellate and the corolla sympetalous, WERNHAM (**22**) lists the following tendencies: (*a*) actinomorphy to zygomorphy; (*b*) reduction in number of fertile stamens; and (*c*) reduction in number of ovules per carpel. The first two tendencies are particularly evident in the Solanaceae.

Material and methods

The plants listed were studied. The genera have been arranged according to

the classification of WETTSTEIN (23) in ENGLER and PRANTL's *Die Natürlichen Pflanzenfamilien:*

Nicandreae	*Nicandra physalodes* Pers.
Solaneae-Lyciineae	*Lycium halimifolium* Mill. *Atropa belladonna* L.
Solaneae-Hyoscyaminae	*Hyoscyamus niger* L.
Solaneae-Solaninae	*Physalis alkekengi* L. (*P. francheti* Hort.) *Capsicum frutescens* L. var. *grossum* Bailey (Early Wonder) *Solanum tuberosum* L. *S. nigrum* var. *guineense* L. *S. melongena* L. var. *esculentum* Nees. *S. pseudocapsicum* L. *S. dulcamara* L. *S. rostratum* Dunal *Lycopersicon pimpinellifolium* Mill. (included under *Solanum* by WETTSTEIN)
Datureae	*Datura stramonium* L. *D. meteloides* Dunal
Cestreae-Nicotianae	*Nicotiana sanderae* Sander (Crimson Bedder) *N. glauca* Graham *Petunia hybrida* Vilm. (Snowstorm-Giant Single) *Nierembergia hippomanica* Miers
Salpiglossideae	*Salpiglossis sinuata* Ruiz and Pav. *Schizanthus pinnatus* Ruiz and Pav.

The flowers and fruits were collected at various stages in development, killed and fixed either in Navashin's solution or in formalin-acetic-alcohol, dehydrated by the usual tertiary butyl-alcohol method, and imbedded in paraffin. Both longitudinal and transverse serial sections were cut at 12 μ, although some of the large fruits were sectioned at 20 μ. The material was then stained in a modified Flemming's triple stain.

Observations

Of the flowers studied, those of Nicandreae, Solaneae, and Datureae follow essentially the same pattern of floral anatomy. The exceptions are *Solanum rostratum*, which appears to be an anomalous form, and *Hyoscyamus niger*, which has slightly irregular flowers. All the other flowers are actinomorphic, the stamens are fertile and of equal size, and there are two adaxial bundles to each carpel. *Physalis alkekengi* has been chosen as typical of this group.

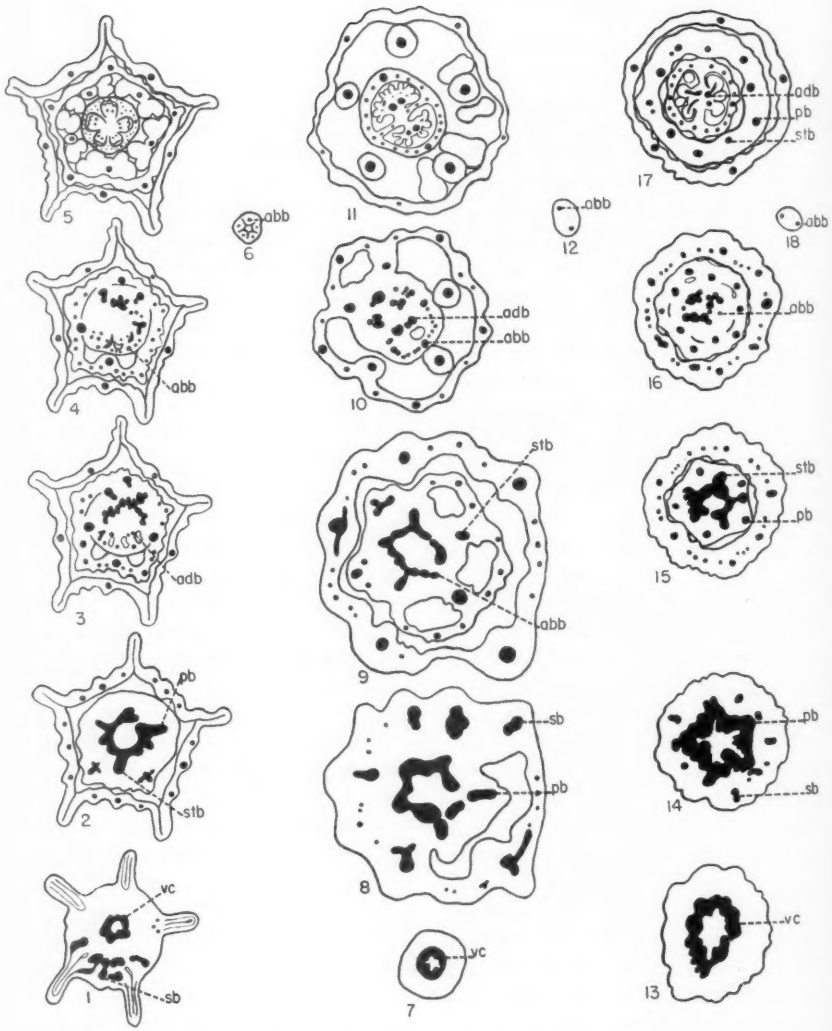
The pedicel of *P. alkekengi* is an amphiphloic siphonostele; in the receptacle the vascular tissue consists of ten distinct bundles (fig. 25). The first five divergences extend outward and are the median sepal bundles. At the periphery, lateral divergences from the sepal bundles are anastomosed with one another (fig. 26). Slightly distal to the main sepal divergences, the vascular cylinder is again continuous. Next there are five petal divergences (fig. 26) alternating with the sepal bundles and accompanied by small parenchymatous gaps. At each side of the main petal bundle there is a lateral divergence, so that the corolla has fifteen prominent bundles in all. The vascular tissue is again continuous at a

level slightly distal to the petal divergences. Opposite the median sepal bundles and alternate with those of the petals, five stamen bundles diverge from the stele without accompanying gaps (fig. 27). After the divergence of the first three sets of floral parts, the vascular cylinder is elliptical and again continuous. In the middle of the long arcs of the ellipse two large bundles (the adaxials) diverge toward the center of the ovary and are accompanied by gaps. At almost the same level the remaining portion of the ellipse is broken into small bundles which diverge outward slightly and then upward. These are bundles of the carpellary walls. The abaxial bundles of the carpels begin at the middle of the two short arcs of the ellipse, diverge outward at a slightly more distal level than the other carpellary bundles, and extend upward the length of the style (figs. 27, 28).

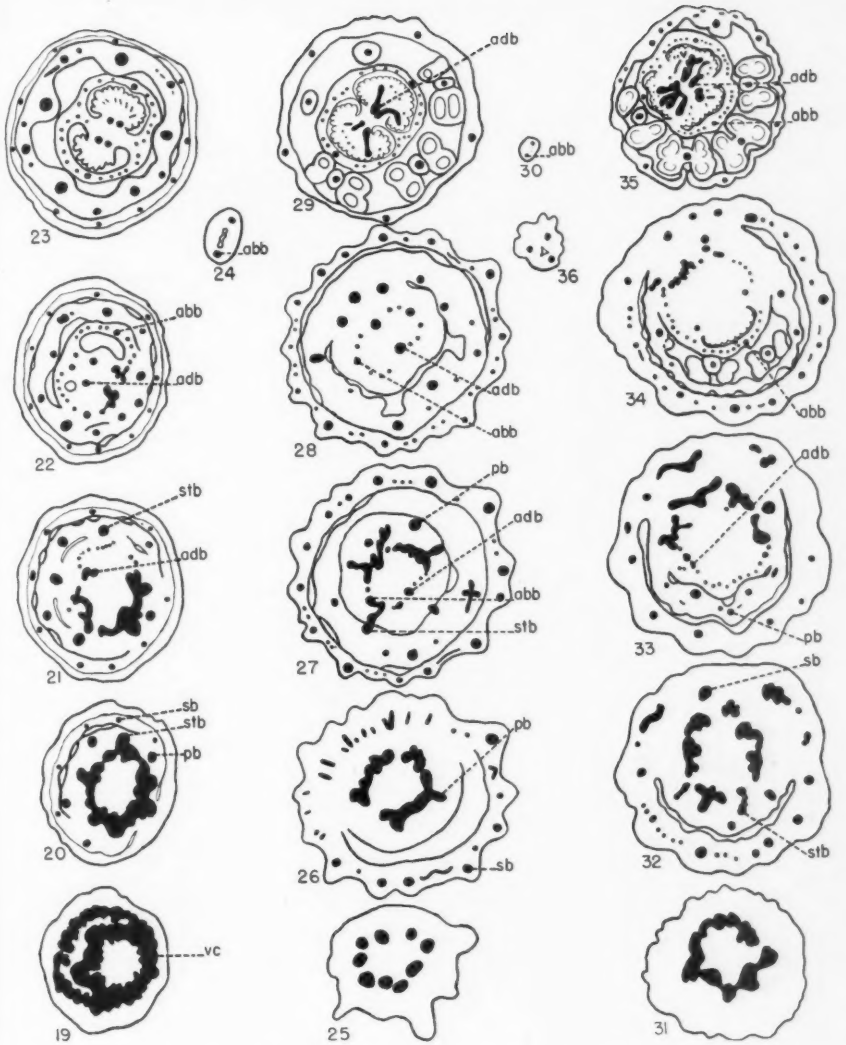
There are usually two carpels in *Physalis*. The abaxial bundle of one carpel is opposite the mid-bundle of a petal, while the other abaxial bundle is opposite a stamen bundle (fig. 29). At the level at which the two adaxial bundles are almost in the center of the ovary, four cavities become evident between the adaxial bundles and those of the carpellary walls. Then each adaxial bundle is divided into two, one for each carpel. The tissue between the two cavities in each carpel or the tissue between the carpel wall and the placenta extends for only a short distance. Distal to this, the ovary consists of the carpel walls, the two loculi, and two massive placentae bearing ovules which are supplied with vascular tissue through divergences from the adaxial bundles (fig. 29). At the top of the ovary the two septa do not meet completely, so that the uppermost portion of the ovary and the base of the style appear to be unilocular. Still farther up the style there is no cavity, the entire central portion being occupied by transmitting tissue. The smaller anastomosing bundles of the carpellary walls and the adaxial bundles are not present in the style, but the abaxial bundles extend to the stigma (fig. 30).

From the standpoint of interpretive morphology it is necessary to note the position of the xylem and the phloem in the adaxial bundles. These bundles diverge from an amphiphloic siphonostele and remain bicollateral until they are divided at the level where the four cavities first become evident. Then the xylem is separated into two groups by parenchymatous rays, phloem is differentiated completely around each group of xylem, and the bundles are amphicribral.

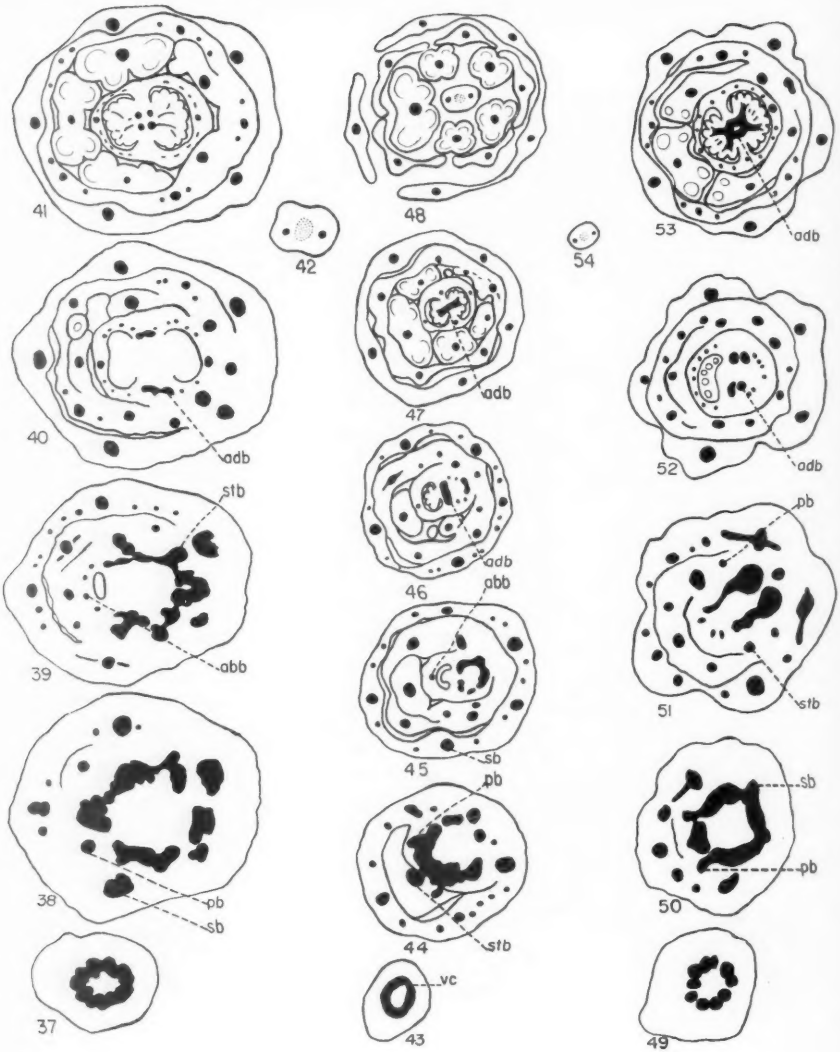
The other genera of Nicandreae, Solaneae, and Datureae follow rather closely the floral anatomy of *Physalis*. There are, however, some differences. In *Lycium halimifolium* the five divergences to the sepals may or may not be accompanied by very small gaps. After these first divergences, the stele is star-shaped. From each of the five points there is a divergence—the lateral sepal bundle. Smaller lateral bundles may be formed either through trifurcation of the main lateral or as divergences from this bundle after it is already differentiated (fig. 14). *Hyoscyamus niger* differs from all other Solanaceae studied in the formation of its sepal bundles. The divergences to the sepals are not separate traces but an entire ring of tissue. Certain portions of this outer cylinder stain darker and become differentiated into five large median, five large lateral, and an indefinite number of small anastomosing bundles. The ring of darker-staining tissue remains in the calyx and becomes lignified in the mature flower (figs. 19–23). *Capsicum frutescens* var. *grossum* and *Solanum melongena* var.



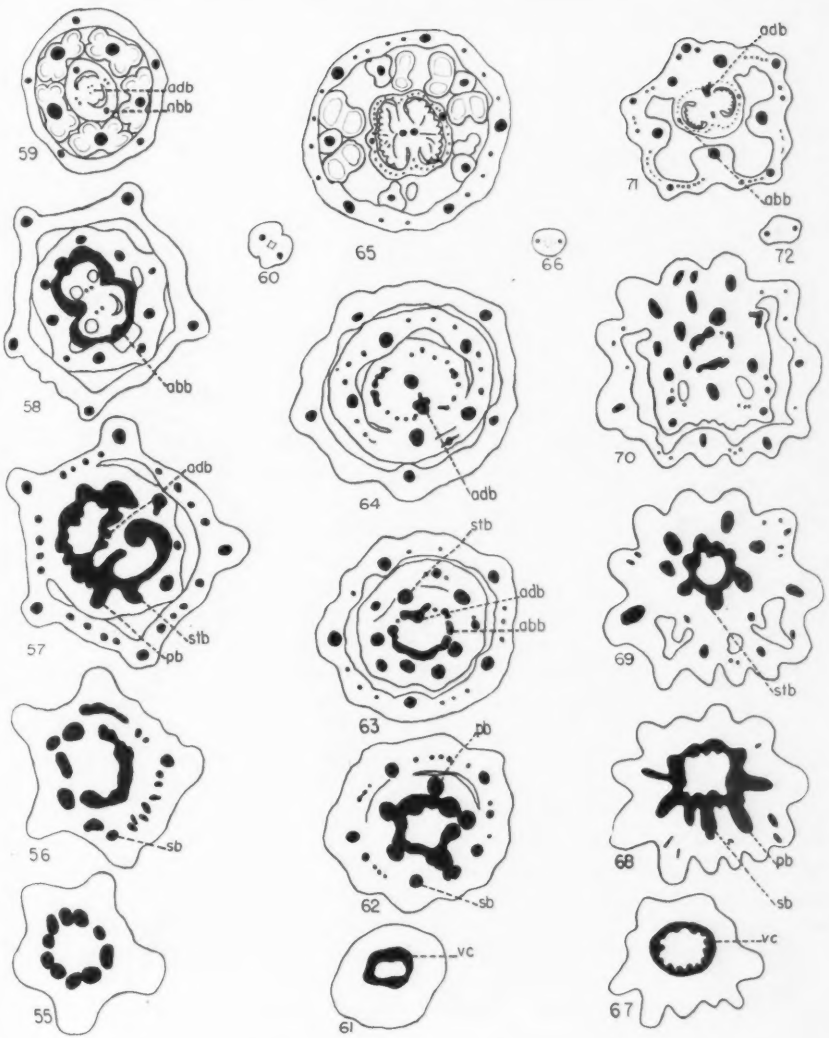
FIGS. 1–18.—Transections of flowers from pedicel to apex showing comparable stages in floral development. Species in figs. 1–90 arranged in phylogenetic sequence. Figs. 1–6, *Nicandra physalodes*. Figs. 7–12, *Atropa belladonna*. Figs. 13–18, *Lycium halimifolium*. Figs. 10, 11, shown without sepals. Figs. 6, 12, 18, style. (*vc*, vascular cylinder; *sb*, sepal bundle; *pb*, petal bundle; *stb*, stamen bundle; *adb*, adaxial bundle; *abb*, abaxial bundle.)



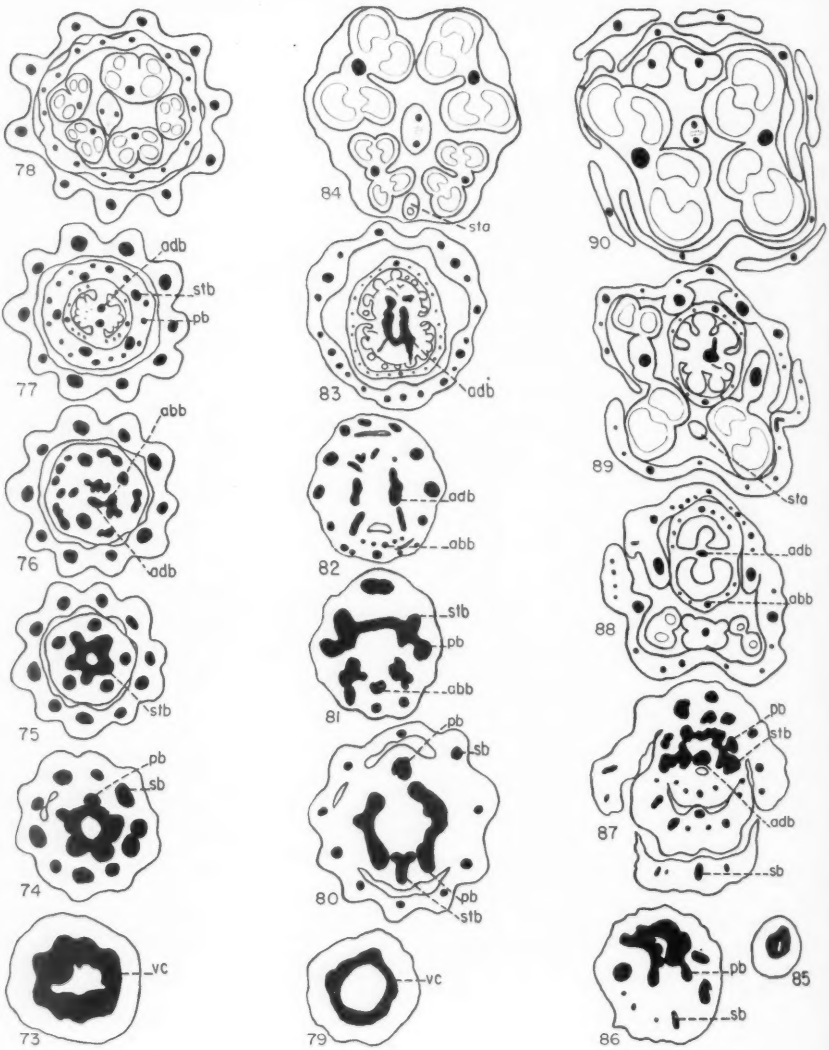
FIGS. 19–36.—Transections of flowers from pedicel to apex. Figs. 19–24, *Hyoscyamus niger*. Figs. 25–30, *Physalis alkekengi*. Figs. 31–36, *Capsicum frutescens* var. *grossum*. Figs. 29, 35, shown without sepals. Figs. 24, 30, 36, style.



FIGS. 37–54.—Transections of flowers from pedicel to apex. Figs. 37–42, *Solanum tuberosum*. Figs. 43–48, *Solanum rostratum*. Figs. 49–54, *Lycopersicon pimpinellifolium*. Figs. 42, 54, style.



FIGS. 55–72.—Transections of flowers from pedicel to apex. Figs. 55–60, *Datura stramonium*. Figs. 61–66, *Nicotiana glauca*. Figs. 67–72, *Petunia hybrida*. Figs. 59, 65, 71, shown without sepals. Figs. 60, 66, 72, style.



FIGS. 73–90.—Transections of flowers from pedicel to apex. Figs. 73–78, *Nierembergia hippomanica.* Figs. 79–84, *Salpiglossis sinuata;* figs. 81–83, shown without sepals; fig. 84, showing only stamens and style. Figs. 85–90, *Schizanthus pinnatus;* figs. 88, 89, shown without sepals. (*sta*, staminodium.)

esculentum are commercial forms. Because cultivation has brought about a multiplication of floral parts, the floral anatomy of these plants differs somewhat from that of the ancestral types. Pepper has six sepals (figs. 32–34), while eggplant is even more variable. In most of the latter there are eight sepal divergences which do not come from the cylinder at the same level. These divergences are in two sets of four each, those of one set alternate with those of the other and at a level slightly distal to the first four. The anastomoses of the sepal bundles in *Datura stramonium* (fig. 56) and *D. meteloides* form an almost continuous ring of tissue between the main sepal bundles.

The differences in petal and stamen divergences require only brief mention. In *Lycium* (fig. 15), *Capsicum* (fig. 32), *Solanum dulcamara*, and *Lycopersicon* (fig. 50) the petal divergences are accompanied by parenchymatous gaps in the vascular cylinder, but no gaps are associated with the stamen divergences in any plant studied. The vascular cylinder does not again become continuous after the petal divergences in *Capsicum* (figs. 32–34). In the cultivated forms of *Solanum melongena* var. *esculentum* and *Datura meteloides* the number of petals and stamens is indefinite, and there is no sharp differentiation between these two floral sets.

The ovary of *Nicandra physalodes* usually consists of five carpels. The abaxial bundle of each carpel is opposite a petal bundle (fig. 4). In the formation of the carpellary vascular system from the stele, there are five outward divergences accompanied by gaps. These divergences are the abaxial bundles. Between each gap the remaining vascular tissue diverges outward and is divided into two strands. Each strand represents an adaxial bundle and several small bundles which are anastomosed through the carpellary tissue with the abaxial bundle. The two adaxial bundles which diverge together are for two carpels whose edges represent a septum common to both (figs. 3–5). When these adaxial bundles diverge they are bicollateral, but at more distal levels they are amphicribral.

In *Lycium halimifolium* a complete siphonostele remains after the stamen divergences (fig. 15). Two bundles opposite each other—the two abaxial bundles—diverge outward slightly and are accompanied by gaps. The remaining portion of the cylinder is broken into four equal arcs, each divided periclinally. The bundles of the carpellary walls diverge from the outer portion of each arc, while the inner parts of the four arcs diverge toward the center of the ovary and are the four adaxial bundles (figs. 16, 17). Since these periclinal divisions of the stele are through the xylem, each adaxial bundle has xylem facing the loculus and phloem centrad to the xylem. At more distal levels the bundles become amphicribral.

The carpel bundles of *Atropa belladonna* are derived from a stele in the form of an ellipse (fig. 9). In the two short arcs two bundles diverge outward slightly and are the two abaxial bundles. From the long arcs of the ellipse two large central bundles on each side diverge inward and are the four amphicribral adaxial bundles (figs. 9–11).

The cultivated variety, *Capsicum frutescens* var. *grossum*, has a three-carpellary ovary. The three abaxial bundles diverge from the vascular tissue centrad to three alternate stamen divergences. The inner portions of the other three stamen divergences form three adaxial bundles. At the level at which the three loculi can first be distinguished, the three adax-

ial bundles are in the septa. Then each adaxial bundle is divided into two, one for each adjacent carpel (figs. 33–35).

The carpellary anatomy of *Solanum tuberosum* (figs. 39–41), *S. nigrum* var. *guineense*, *S. pseudocapsicum*, *S. dulcamara*, and *Lycopersicon pimpinellifolium* (figs. 51–53) is similar to that of *Physalis*. In *S. nigrum* and in *L. pimpinellifolium* (figs. 53) the four adaxial bundles form an almost continuous cylinder of vascular tissue. No attempt has been made to interpret the ovary of *S. melongena* var. *esculentum*. Such an explanation is difficult without access to the ancestral type. In the large fleshy gynecia of the cultivated forms the carpellary tissue is very much proliferated and somewhat distorted. The number of carpels is indefinite. Ordinarily there are five to six around a thick central mass of tissue, in which one to two additional carpels may be found. The latter may constitute another set of carpels produced from the axis, which has remained meristematic. Such additional sets of carpels are found in the navel orange, in many "double" flowers, and are not uncommon in the Solanaceae, having been observed in both pepper and tomato. The vascular supply to the carpels of eggplant is derived from the siphonostele remaining after the stamen divergences. At a level just proximal to the formation of each carpel, the two adaxial bundles and the bundles of the carpellary wall diverge inward, so that the position of each carpel becomes first delimited by vascular tissue which is horseshoe-shaped. In the mature carpel there are two semicircles of vascular bundles around each carpel, and the ovules are borne on the placenta and also on what appears to be the carpel wall.

At the level at which the petal and stamen bundles diverge outward in *Datura stramonium*, there are two inward divergences which are directly opposite each other, are accompanied by gaps, and are divided to form four strands of vascular tissue (fig. 57). These inward divergences are the four adaxial bundles, which differentiate as amphicribral bundles in the center of the ovary. After these divergences a continuous cylinder of meristematic tissue is formed (fig. 58). From it are differentiated two abaxial bundles, many small bundles in the carpellary walls, and two somewhat larger and distinct bundles in each of the septa (fig. 59). These last four bundles are probably not different from the bundles in the carpellary walls, but they seem to be much more distinct than the anastomosing bundles in *D. stramonium* and extend up into the basal part of the style. The lower half of the ovary and the fruit appears to have four carpels, resulting from the extension of tissue from the wall of each carpel to the placenta opposite the abaxial bundle. There are no vascular bundles in this tissue, however, and the structure seems to have no morphological significance.

The floral anatomy of the anomalous form, *Solanum rostratum*, resembles most closely that of *Schizanthus pinnatus*. The flowers show a marked tendency toward zygomorphy, with the large lower sepal and stamen differentiating before the other floral parts (fig. 44). This stamen is twice as large as the others (fig. 48). After the divergences to the first three floral sets, the vascular cylinder is divided into two abaxial, two prominent adaxial, and smaller bundles in the carpellary walls (fig. 45). The two adaxial bundles diverge from the periphery of the ovary to its center and fuse to form one adaxial bundle for the two carpels (figs. 46, 47). When the adaxial bundles are diverging, they are bicollateral; after they fuse, the

one bundle is amphicribral. *Solanum* is an extensive genus, and it is possible that evolution has taken place within the genus itself.

While the floral anatomy of the genera studied in Cestreae-Nicotianae and Salpiglossideae is basically like that of *Physalis alkekengi*, individual descriptions are necessary to explain the differences.

After the divergence of the two abaxial bundles in *Nicotiana sanderae*, the remaining carpellary tissue consists of a bundle adjacent to each abaxial bundle and two long segments of vascular tissue opposite each other and on alternate radii from the abaxial bundles. From the middle of each segment an adaxial bundle diverges toward the center of the flower and is divided. There are two amphicribral adaxial bundles for each carpel. In *N. glauca* the two adaxial bundles diverge toward the center of the ovary (figs. 63, 64). For a short distance they are fused to form one double bundle. Distal to this there is one adaxial bundle in the placenta of each carpel (fig. 65).

Each of the five sepal divergences of *Petunia hybrida* trifurcates to form one median and two lateral sepal bundles (fig. 68). These sepal divergences are not accompanied by parenchymatous gaps, but small gaps may be associated with the petal divergences. While all the stamens are fertile, one is smaller than the other four The cylinder remaining after the stamen divergences is divided into four segments—two large ones opposite each other and two small ones on alternate radii (figs. 69, 70). An abaxial bundle and two bundles for the carpellary wall are derived from each small segment. The large one is broken into several bundles for the carpellary wall and an adaxial bundle which diverges toward the center of the flower. Each adaxial bundle may be double or divided into two bundles (fig. 71). In the mature ovary the adaxial bundles are amphicribral.

In *Nierembergia hippomanica* there are ten sepal divergences, which are not accompanied by gaps. Five of these are the main sepal bundles and five are laterals (fig. 74). When the calyx is separated into lobes, the laterals form a dichotomy with one branch to each adjacent lobe. The petal and stamen bundles are formed in the usual manner (figs. 74, 75). One to three of the five stamens are larger, but all are fertile (fig. 78). The last two stamen bundles to be diverged are opposite the two abaxial bundles. The remaining vascular tissue consists of two segments on opposite radii from the abaxial bundles. From these are diverged the two large adaxial bundles and part of the bundles of the carpellary walls (figs. 76, 77). The remaining bundles of the carpellary walls are lateral divergences from the abaxial bundles. Toward the distal end of the ovary the two adaxial bundles form one bundle, which is then divided into many small ones extending to the base of the style.

Salpiglossis sinuata shows a marked tendency toward zygomorphy and reduction in the number of fertile stamens. There are ten divergences to form five median and five lateral sepal bundles, as in *Nierembergia* (fig. 80). Only three of the ten bundles are accompanied by gaps. One of these is the sepal bundle in the lower lip opposite the staminodium. The other two such bundles are in the upper lip opposite the two large fertile stamens. After the sepal divergences the vascular tissue is usually continuous, although this state is difficult to observe because the lower lip of the flower differentiates at a more proximal level than the upper

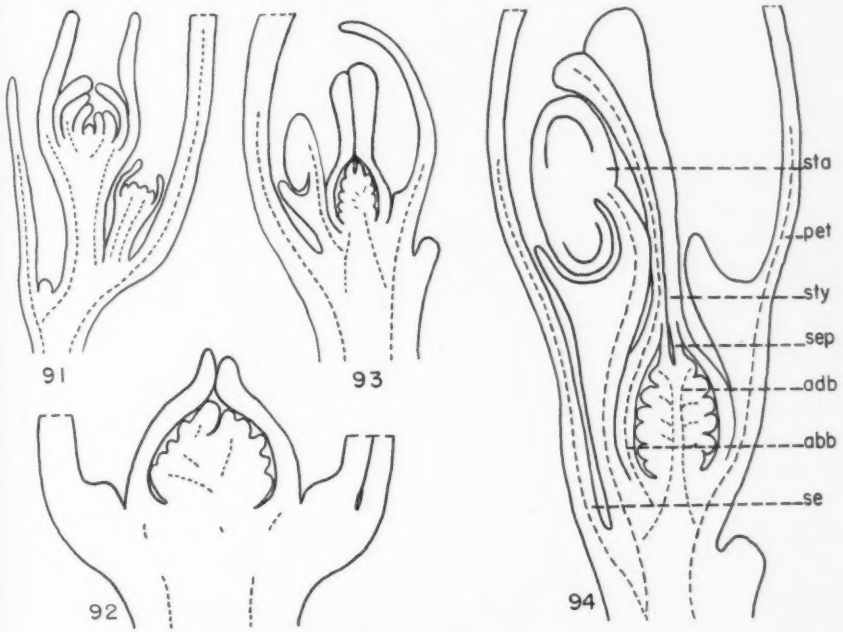
part (fig. 81). The petal divergences are not accompanied by gaps. At the level at which the calyx is just diverged from the receptacle, the following bundles can be distinguished in the lower lip: three stamen, two petal, and an abaxial. At the same level the upper lip of the flower consists of one large petal bundle in the uppermost part and a semicircle of vascular tissue toward the center (fig. 81). When the bundles in the lower lip become differentiated, the remaining petal and stamen bundles diverge from the semicircle of vascular tissue in the upper part. There are two large fertile stamens, two small fertile ones, and a staminodium (fig. 84). Two strands of vascular tissue opposite each other remain between the lower and the upper parts of the flower (fig. 82). These diverge to the center and are the two adaxial bundles. At more proximal levels the adaxial bundles appear to be double, but at distal levels and in mature flowers the adaxial bundles are single and amphicribral, with only a slight tendency for the xylem to be separated into two groups by parenchymatous rays. At some levels the two adaxial bundles form an almost complete ring of vascular tissue (fig. 83).

Of all the plants studied, *Schizanthus pinnatus* is the most advanced. It is obviously zygomorphic, and the fertile stamens are reduced to two. In its floral anatomy, however, it still follows the same basic plan of all Solanaceae. The first divergence, the sepal bundle in the lower lip, is not accompanied by a gap. Slightly distal to this first sepal divergence and on either side of it are two petal divergences not accompanied by gaps (fig. 86). These petal divergences trifurcate; the two outer bundles are the lateral ones of the sepals, while the middle one is the petal bundle. Opposite the first sepal bundle there is a divergence to the most rudimentary of the staminodia. Only the filament of this stamen is formed, and there is rarely any indication of an anther (fig. 88). The divergences to the upper part of the flower are formed in the same manner but depart at more distal levels (fig. 87). The two stamens on either side of the vestigial one are fertile, and their anthers are very large in proportion to the other floral parts (fig. 90). The two uppermost staminodia develop small sterile anthers. After the stamen divergences, the vascular cylinder becomes solid. One to several cells in the center do not stain so dark as the surrounding meristematic vascular tissue. The outer part of this solid vascular cylinder is broken into a circle of small bundles which diverge outward and become the two abaxial bundles and the bundles of the carpellary walls. One abaxial bundle is directly opposite the most rudimentary stamen, while the other is between the two sterile stamens (fig. 88). The inner portion of the vascular cylinder does not change position but becomes the one adaxial bundle common to the two carpels (figs. 88, 89). At the level at which the ovules and placentae appear, one to several vessels become differentiated in the middle of the adaxial bundle. These vessels are the same in number as the large parenchymatous cells in the solid cylinder when it is first formed. The adaxial bundle is single, with no evidence of doubling, but at the top of the ovary this bundle may be divided.

The genera of Solanaceae can be separated into two groups according to the types of fruits produced. *Nicandra*, *Lycium*, *Atropa*, *Physalis*, *Capsicum*, *Solanum*, and *Lycopersicon* form berries, while *Hyoscyamus*, *Datura*, *Nicotiana*, *Nierembergia*, *Petunia*, *Salpiglossis*, and *Schizanthus* produce capsules. It is inter-

esting to note that, with the exception of *Hyoscyamus*, the more simple flowers develop into berries, while the more complex ones form capsules. Only in the first group is there much anatomical change from ovary to fruit.

In the fruit of *Capsicum frutescens* var. *grossum* the placentae are very much enlarged, but this increase in size does not keep pace with the upward and outward growth of the pericarp and the septa. The placental tissue occupies a relatively small part of the loculi in the lower half of the fruit and is no longer fused with the lower portion of the style. The stylar end of the fruit is unilocular, since the septa are attached only to the pericarp. In *Solanum nigrum* var. *guineense* and in *S. pseudocapsicum* the parenchyma of both the pericarp and the placentae proliferates and almost incloses each seed, but the tissues never actually fuse. The fruit of *S. melongena* var. *esculentum* is produced by enormous growth of the parenchyma of the placentae. The placental tissue of *Lycopersicon pimpinellifolium* proliferates greatly and almost entirely incloses each of the seeds.



FIGS. 91–94.—Successive stages in floral development. Figs. 91, 93, 94, *Nierembergia hippomanica*. Fig. 92, *Physalis alkekengi*. (*se*, sepal; *sep*, septum; *sty*, style; *pet*, petal.)

In floral development the Solanaceous flowers included in this paper follow closely the descriptions of COOPER (4) and WARNER (21) for *Lycopersicon*, URQUHART (17) for *Lycium*, COCHRAN (3) and AUGUSTIN (2) for *Capsicum*, and YOUNG (25) for *Solanum tuberosum*. The sepals, petals, stamens, and carpels are formed in acropetal succession (fig. 91). While in longitudinal section the ovule-bearing tissue of the gynecium appears to be a prolongation of the axis, actually it

is axis plus carpellary tissue. The outer portions of the two carpels arch over the central axile prolongation and finally fuse (fig. 92). Continued elongation of the outer portions of the two carpels forms the style (fig. 93). At the apex of the central prolongation there is a depression, on either side of which the tissue grows upward and fuses with the basal part of the style (figs. 92–94). SATINA and BLAKESLEE (14) describe this occurrence in *Datura*. In the description of *Physalis alkekengi*, at the top of the ovary only the septal tissue remains. Finally the two septa are separated. The tissue which grows upward to fuse with the style is then the tissue of the two septa. The fusion of the septal tissue with the style differs somewhat in the various species studied. In *Hyoscyamus*, *Physalis*, *Capsicum*, *Nierembergia*, *Salpiglossis*, and *Schizanthus* ovules are formed before this fusion takes place, while in *Datura*, *Nicotiana*, and *Petunia* this fusion precedes the development of ovules.

Discussion

The syncarpous ovary, according to the Candollean theory, results from the fusion of carpellary leaves. The ovules are borne on the undiverged margins of these leaves, and the axis, if present, serves only as a support for these structures. There are many arguments for and against this theory. Before discussing them, it might be well to define the terminology used in carpel morphology.

The two principal types of placentation are axile and parietal. These terms generally apply only to position and have no morphological significance. Parietal placentation results when the ovules are borne on projections from the carpel wall. When the carpels fuse in the center of the ovary and divide it into as many loculi as there are carpels, the placentae are axile. PAYER (12), STRASBURGER (16), and GRAY (7) are among those who state that with few exceptions all syncarpous, plurilocular ovaries have axile placentation. "Axial" and "cauline" refer to structures which are morphologically stem, while "foliar" applies to leaflike parts. The confusion in the literature arises from the use of positional terms in a morphological sense.

Among those who disagree with DE CANDOLLE and adhere to the axial theory are PAYER (12), SCHLEIDEN (15), and ST. HILAIRE (WORSDELL, 24). PAYER states that the "axile part of a syncarpous ovary constitutes the placentae and bears the ovules, while the carpellary leaves constitute the walls of the ovary." SCHLEIDEN states: "In all families with plurilocular ovaries one can easily follow the origin of the placentae which come from the axis." ST. HILAIRE also thinks that "the placenta is a continuation of and represents the axis." Between the axial and the appendicular theories is HAYWARD (9), who explains: "In the axile type of placentation, the placental tissue lies at the center of the ovary and may be foliar, or in part foliar and in part cauline."

Outstanding followers of the Candollean theory are VAN TIEGHEM (18, 19), HENSLOW (10, 11), EAMES (5), and ARBER (1). VAN TIEGHEM and HENSLOW are particularly helpful because they give definite criteria for interpreting the nature of the placentae. The following quotations are taken from HENSLOW. The first one (10) explains the views of VAN TIEGHEM, while the second one (11) gives the ideas of HENSLOW on the subject.

Van Tieghem in his investigations upon the anatomy of the pistil regarded the circle of vascular cords which have their trachae on the inner side, i.e. facing the medulla, and the

phloem on the outside of it, i.e. facing the cortex, as indicating the axis; but when the circle is broken up and these two elements of the cords are altered or reversed in position as they often are in the placentas of a carpel, he pronounced them to be "foliar."

The point, then, at which carpellary cords branch off from the common stem in the first case may be regarded as marking the termination of their axial character; and in the latter case, at the separation of the parts of the "horse-shoes" to form groups of threes. With regard to those cords which become marginal and placentary, it is important to notice the position of their spiral vessels. If they are situated on the side of the cord nearest the medulla, the cord may generally be regarded as axial; if they are on the other side, i.e. nearest the ovary-cell, and if in transverse sections they exhibit intermediate positions, in which they are central or scattered irregularly within the phloem, they are then marginal and placentary.

When floral parts are undiverged, the vascular anatomy seems to be the most valuable criteria for determining the origin of the parts involved. In this paper, therefore, the interpretation of VAN TIEGHEM and HENSLOW is used. If the adaxial bundles retain their stem-like character, with the xylem facing the center of the flower, the structures will be considered as cauline; if the xylem is facing the loculus, or if the bundles are amphicribral, the structures will be considered as foliar.

As for the literature on the Solanaceae, PAYER (12), VIDAL (20), and RENDLE (13) describe the placentae as axile. In *Lycopersicon esculentum* var. *cerasiforme*, WARNER (21) explains the placentae as follows: "Apparently it is from the central more or less columnar axis that ovules arise. Thus they may be thought of as cauline in origin, or perhaps, the surface of this axis may be regarded as an undiverged portion of the sporophyll, in which case the ovules would be regarded as foliar." URQUHART (17), working with *Lycium halimifolium*, states: "Although the placental structure is at this stage continuous with the carpellary tissue, it is largely cauline in origin. The ovules may be regarded as arising from cauline, axile placentae rather than foliar ones."

VIDAL (20) studied several species of the Solanaceae and concluded that in all of them the axis forms a thick septum raised to the summit of the ovary. In summarizing his work, VIDAL states: "The Solanaceae are characterized by a very well developed, parenchymatous axis raised to the summit of the ovary."

The most comprehensive paper on floral anatomy in the Solanaceae is by GRELOT (8). His emphasis is somewhat different from the present work, but on the whole the results agree. Concerning the adaxial bundles, he says: "If a certain number of bundles converge toward the center of the placentae and unite side by side to form a cylinder, the latter delimits a true pith whose role is different from that of the exterior parenchyma of the cylinder. One perceives then that the bundles take the same orientation as that in the stem." SATINA and BLAKESLEE (14), who worked with periclinal chimeras obtained in *Datura*, state: "The initiation and development of the carpel wall, septa, and placentae suggest that they are axial and not foliar in origin."

Of a rather different opinion is GOEBEL (6). He describes the development of the carpels as follows:

> The process is exactly the same where we have in each loculus a many-ovuled placenta developed as in Solanaceae and Scrophulariaceae. The ovary in its upper part is unilocular with two parietal placentas. . . . . The carpels use up entirely the torus, and form to a certain extent a double sole, the septal wall. The margin of the cup of the ovary shows an increased growth at the points corresponding to

the apices of the carpels and the lateral parts raise themselves somewhat at the position of concrescence and there form the parietal part of the placenta. Beyond this the question of how far the flower-axis is drawn into the formation of the ovary is of quite subordinate importance.

The floral anatomy of the Solanaceae demonstrates that the placentae are usually be distinguished. GRELOT's contention that such a cylinder is proof of the cauline nature of the placental bundles seems unfounded. The carpellary walls, the septa, and the ovule-bearing portions of the placentae are foliar in origin. The central fleshy part of the ovary is parenchymatous stelar tissue.

TABLE 1

Species	Type of flower	Stamens	Adaxial bundles	Fruit
Nicandra physalodes Atropa belladonna Lycium halimifolium	Actinomorphic	All fertile	Two per carpel	Berry
Hyoscyamus niger	Slightly zygomorphic	All fertile	Two per carpel	Capsule
Capsicum frutescens Solanum nigrum S. melongena S. dulcamara S. pseudocapsicum S. tuberosum	Actinomorphic	All fertile	Two per carpel	Berry
Solanum rostratum	Zygomorphic	1 large, 4 small } fertile	One for 2 carpels	Berry
Lycopersicon pimpinellifolium	Actinomorphic	All fertile	Two per carpel	Berry
Datura stramonium D. meteloides	Actinomorphic	All fertile	Two per carpel	Capsule
Nicotiana sanderae	Slightly zygomorphic	All fertile	Two per carpel	Capsule
N. glauca	Slightly zygomorphic	All fertile	Two for 2 carpels	Capsule
Petunia hybrida	Slightly zygomorphic	4 large, 1 small } fertile	Two double bundles for 2 carpels	Capsule
Nierembergia hippomanica	Slightly zygomorphic	1–3 large, 4–2 small } fertile	Two for 2 carpels	Capsule
Salpiglossis sinuata	Zygomorphic	2 large, 2 small } fertile 1 sterile	Two for 2 carpels	Capsule
Schizanthus pinnatus	Zygomorphic	2 large fertile 2 small sterile 1 vestigial	One for 2 carpels	Capsule

foliar in origin. In all the species studied the adaxial bundles in the older flowers and fruits are amphicribral and accompanied by gaps in the vascular cylinder. This applies even to *Schizanthus pinnatus* and *Solanum rostratum*, which have only one adaxial bundle. The occurrence of the adaxial bundles as a cylinder in the center of the ovary was noted in *Solanum nigrum*, *Lycopersicon pimpinellifolium*, and *Salpiglossis sinuata*, but the vascular tissue does not resemble an amphiphloic siphonostele and the individual amphicribral bundles can

In the Solanaceous flower the margins of the carpels are undiverged from the stem. At first there is conjoint growth of stem and carpels, but in the more mature flower the apical region of the stem ceases its meristematic activity. The exception is found in such plants as tomato, eggplant, and pepper, where growth continues to form a second fruit. Just how much of the ovule-bearing prolongation of the ovary is cauline and how much foliar would be difficult to prove. The adaxial bundles are foliar, and in longitudinal section they probably limit the

inner bounds of the carpels. The placentae are axile in position and foliar in origin. The Solanaceous flowers described in this paper can best be interpreted by the appendicular theory of DE CANDOLLE.

In general, the floral anatomy furnishes additional evidence of the validity of WETTSTEIN's arrangement of the genera. It would seem better to place the Hyoscyaminae at the end of the Solanaceae because the flowers of *Hyoscyamus* are slightly irregular and the fruit is a capsule. Among the Cestreae-Nicotianae and the Salpiglossideae there is a gradual reduction in the number of adaxial bundles per carpel. The phylogenetic tendencies of the Solanaceae described in this paper are summarized in table 1.

Summary

The floral anatomy of fourteen genera and twenty-one species of the Solanaceae was examined. The arrangement of the genera is according to WETTSTEIN in ENGLER and PRANTL's *Die Natürlichen Pflanzenfamilien*. In the order of their complexity, these genera include *Nicandra*, *Lycium*, *Atropa*, *Hyoscyamus*, *Physalis*, *Capsicum*, *Solanum*, *Lycopersicon*, *Datura*, *Nicotiana*, *Petunia*, *Nierembergia*, *Salpiglossis*, and *Schizanthus*. The following evolutionary tendencies were noted: actinomorphy to zygomorphy; reduction of fertile stamens from five to two; and reduction in the number of adaxial bundles from two per carpel to one for two carpels. Despite these variations, the floral anatomy follows the same basic plan in all the species studied. The siphonostele generally becomes continuous after the divergences to each of the first three floral sets, and the carpellary bundles are formed from the remaining vascular tissue. Because the adaxial bundles are amphicribral and accompanied by gaps in the vascular cylinder, they are foliar rather than cauline. The placentae are axile in position, while the carpel walls, the septa, and the ovule-bearing portions of the placentae are foliar in origin. The central parenchymatous part of the ovary is stelar, and the carpels are undiverged leaves.

The writer is indebted to ANNA PEDERSEN KUMMER of Chicago for the seeds of *Datura stramonium* and *Solanum rostratum*, to GERTRUDE HOFFERT of Hayden, Arizona, for the seeds of *Nicotiana glauca*, and to Dr. D. M. CROOKS of the Bureau of Plant Industry for the seeds of *Hyoscyamus niger* and preserved material of *Atropa belladonna*. Gratitude is also due Dr. J. M. BEAL and other members of the Department of Botany at the University of Chicago for the many helpful suggestions and advice.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS

LITERATURE CITED

1. ARBER, AGNES, The interpretation of the flower: a study of some aspects of morphological thought. Biol. Rev. **12**:57–84. 1937.
2. AUGUSTIN, BÉLA, Historisch-kritische und anatomisch-entwicklungsgeschichtliche Untersuchungen über den Paprika. Nemetbogsan. 1907.
3. COCHRAN, H. L., A morphological study of flower and seed development in pepper. Jour. Agr. Res. **56**:395–419. 1938.
4. COOPER, D. C., Anatomy and development of tomato flower. BOT. GAZ. **83**:399–411. 1927.
5. EAMES, ARTHUR J., The vascular anatomy of the flower with refutation of the theory of carpel polymorphism. Amer. Jour. Bot. **18**:147–188. 1931.
6. GOEBEL, K., Organography of plants. Part II. Oxford. 1905.
7. GRAY, ASA, The elements of botany. Amer. Book Co., New York. 1887.

8. GRELOT, P., Recherches sur le système libéroligneaux floral Gamopétales bicarpellées. Ann. Sci. Nat. Sér. VIII. 5:1–154. 1897.
9. HAYWARD, H. E., The structure of economic plants. Macmillan, New York. 1938.
10. HENSLOW, G., On the vascular systems of floral organs and their importance in the interpretation of the morphology of flowers. Jour. Linn. Soc. Bot. 28:151–197. 1891.
11. ———, The origin of floral structures through insect and other agencies. Appleton, New York. 1888.
12. PAYER, J. B., Eléments de Botanique. Vol. I: Organographie. Langfois et Leclerq, Paris. 1857.
13. RENDLE, A. B., The classification of flowering plants. Vol. II: Dicotyledons. University Press, Cambridge. 1925.
14. SATINA, SOPHIA, and BLAKESLEE, A. F., Periclinal chimeras in *Datura* in relation to the development of the carpel. Amer. Jour. Bot. 30:453–462. 1943.
15. SCHLEIDEN, M. J., Sur la signification morphologique du placentaire. Ann. Des. Sci. Nat. Sér. 2. Vol. XII. 1839.
16. STRASBURGER, E., Textbook of botany. Macmillan, New York. 1921.
17. URQUHART, BETTY A., The floral development and vascular anatomy of *Lycium halimifolium*. Unpublished Master's Thesis. Univ. Chicago. 1943.
18. VAN TIEGHEM, P., Recherches su · la structure du pistil. Ann. Sci. Nat. Sér. V. 12:127–226. 1868.
19. ———, Recherches sur la structure du pistil et sur l'anatomie comparée de la fleur. Imprimerie Nationale, Paris. 1868.
20. VIDAL, L., Recherches sur le sommet de l'axe dans la fleur des Gamopétales. These. Paris. 1900.
21. WARNER, E. E., The floral development and vascular anatomy of the flower of *Lycopersicon esculentum* var. *cerasiforme*. Unpublished Master's Thesis. Univ. Chicago. 1933.
22. WERNHAM, H. F., Floral evolution: with particular reference to the sympetalous dicotyledons. New Phytol. 10:293–305. 1911.
23. WETTSTEIN, R., Solanaceae. Die Natürlichen Pflanzenfamilien. (A. ENGLER and K. PRANTL.) Leipzig. 1895.
24. WORSDELL, W. C., The structure and morphology of the "ovule." Ann. Bot. 18:57–86. 1904.
25. YOUNG, W. J., The formation and degeneration of germ cells in the potato. Amer. Jour. Bot. 10:325–335. 1923.

INVESTIGATIONS ON RUBBER-BEARING PLANTS: I. PROPAGATION OF TARAXACUM KOK-SAGHYZ BY MEANS OF LEAF CUTTINGS[1]

PAUL R. GORHAM AND MARGARET L. LANDES[2]

Introduction

Interest in obtaining polyploid derivatives from diploid clones of *Taraxacum kok-saghyz* Rod. prompted an experiment to propagate leaves from colchicine-treated root cuttings which gave indications of possessing an increased number of chromosomes. Since material from this source was limited, it was decided to carry out a series of preliminary experiments using diploid leaves. In a comparison of propagation by means of root, crown, and leaf cuttings, MYNBAYEV (3) found the last method too unsatisfactory to warrant further consideration. However, BOWDEN (1) succeeded in obtaining a few plants from leaf cuttings of the Russian dandelion. NAVASHIN and GERASSIMOVA (5) reported that diploid and tetraploid leaves from this plant can be propagated, but in the absence of substantiating data it was not apparent what degree of success might be expected.

HAGEMANN (2) has reviewed, up to 1932, most of the extensive literature on propagation by means of leaf cuttings, while SWINGLE (7) has summarized publications up to 1940. Many of the original papers report results that are frequently inconclusive because of the small num-

[1] Contribution no. 826, Botany and Plant Pathology, Science Service, Ottawa, Canada.

[2] Agricultural Assistants.

bers of leaves used. As HAGEMANN emphasizes, negative results do not necessarily mean that propagation by this method is impossible. A few of these papers contain references to other *Taraxacum* species. HAGEMANN planted eighteen leaves of *T. officinale*, all of which died within 27 days without regenerating roots or shoots. STINGL (6) used forty leaves of *T. officinale* Weber, but these died within 3 days. NAYLOR (4) reported negative results with *T. laevigatum* (Willd.) DC.

Material and methods

For the experiments relating to the temperature of the cutting-frame and treatment with KNO_3, leaves were taken in January from clones of vigorously growing young root cuttings started in flats 2 months before. The material used in the comparison of regeneration of leaves from young and mature plants is described with the experiment. Plants used in the tests with indole-3-butyric acid, naphthaleneacetic acid, asparagine, glycine, *p*-aminobenzoic acid, and glucose were chosen early in March from several clones which had been propagated by means of root cuttings 6–8 months before. These plants had reached 5-inch pot size and had one or two rosettes with twenty to thirty leaves each. The leaves of *Taraxacum latilobum* DC. were taken in April from a plant brought into the greenhouse 8 months before from the collection of Arctic dandelions at the Dominion Arboretum and Botanic Garden, Ottawa.

Young tender leaves in the center of the crown and old yellowed leaves at the periphery were found to root with difficulty and therefore were not tested. With the exception of the treatments employing talc dust, leaves were removed from the parent plant by pulling them away from the crown under water. After rubbing the base between the fingers to remove, as far as possible, any adhering crown tissue, the leaves were stood in the aqueous test solutions. After a stated period they were set in sand in a greenhouse cutting-frame and clipped to a height of 3 inches, in order to reduce transpiration. The temperature of the cutting-frame was maintained at approximately 68°F. The glass top of the frame was covered with paper to screen out direct sunlight. Relative humidity was maintained at a moderate level by frequent watering. The top was slightly raised at night, fully raised during the day. Survival after 10 days was noted and dead leaves were discarded. After 20 days, and again after 30 days, rooted leaves were potted in equal parts sand and manurial compost in $2\frac{1}{2}$-inch pots and placed on a bench in the same house. All unrooted leaves were then discarded. The base of the rooted leaf was planted $\frac{1}{2}$ inch or less in depth, since it was found that shoots form readily only when the leaf bases are just below the soil line. This confirms a similar observation made by NAVASHIN and GERASSIMOVA (5).

Experimentation

The advantages of removal of leaves under water over removal in air is demonstrated by table 1. Excessive wilting is obviated, and improved survival during the first 10 days as well as improved rooting and regeneration result.

Four hundred and fifty leaves from ten clones were divided equally among the shaded cutting-frame at 68°F. as described and two electrically heated frames regulated at 72° ± 2°F., one covered and the other uncovered. At the higher temperature all the leaves died in the covered frame within 10 days and in the uncovered frame within 15 days. At

the lower temperature fifteen leaves in two clones rooted.

Roots of two clones grown in the field for one season from root cuttings were potted in manurial compost December 1. Simultaneously, root cuttings from the same material were started in flats in equal parts of sand, compost, and peat.

TABLE 1

EFFECT OF REMOVAL OF LEAVES IN AIR COMPARED WITH REMOVAL UNDER WATER. TOTALS ARE FROM CONTROL LEAVES OF TWO CLONES COMMON TO TWO DIFFERENT EXPERIMENTS

TREATMENT	NO. LEAVES	PERCENTAGE			
		Survival 10 days	Rooted 20 days	Rooted 30 days	Regenerated 90 days
Air	32	53	9	13	0
Water	64	67	25	38	13

After 53 days the potted plants had abundant leaves and were in full bloom, without fruit. The root cuttings had numerous smaller leaves, with flower buds appearing. The regenerative capacities of leaves from young and from mature plants of these two clones were compared. Root cuttings of clone 93.21 had a higher percentage regeneration than root cuttings of clone 93.38, but this difference was not evident in the regeneration of the leaf cuttings (table 2). Initial survival of leaves from young plants is somewhat better than that of leaves from mature plants, but the production of roots and shoots does not differ appreciably.

Sixty leaves from three clones had $\frac{1}{8}$ inch removed from the base by cutting under water with a razor. These basally clipped leaves regenerated to the same extent as the controls. The leaf tips of 170 leaves from three clones were tested also. The initial survival of these tips was low and all were dead within 20 days.

THIMANN and POUTASSE (8) found that treatment with KNO_3 improved rooting of *Phaseolus* leaves by supplying nutrient to the severed blade. Leaves of *T. kok-saghyz* from eight clones, treated with KNO_3 at a concentration of 500 mg. per liter, had a slightly lower percentage regeneration after 70 days than had the controls (table 3). Roots produced by the treated leaves were only two-thirds as long as those produced by the untreated leaves.

Indole-3-butyric acid in talc, at concentrations of 1000, 2000, and 5000 p.p.m., was tested for its effects on regeneration by dipping the bases of the leaves from each of five clones in the

TABLE 2

COMPARATIVE REGENERATIVE ABILITY OF LEAVES FROM ACTIVELY GROWING YOUNG AND MATURE PLANTS

CLONE	REGENERATION OF ROOT CUTTINGS (%)	TYPE	NO. LEAVES	PERCENTAGE			
				Survival 10 days	Rooted 20 days	Rooted 30 days	Regenerated 90 days
93.21	89	Mature	258	83	19	19	5
		Young	206	88	19	23	1
93.38	35	Mature	82	50	11	11	4
		Young	48	71	21	21	4

dusts just prior to planting. There were slight indications of toxicity at 5000 p.p.m. and of improved regeneration at 1000 and 2000 p.p.m. (table 4).

Naphthaleneacetic acid at a concentration of 50 mg./l., and a solution containing 250, 3, and 1 mg./l. of asparagine, glycine, and *p*-aminobenzoic acid, respectively, were both tested by standing leaves from each of four clones in the solutions for 3 hours prior to planting. Although there was lowered regeneration following the first treatment and improved initial survival and rooting following the second (table 5), natural variability might well account for differences of this magnitude.

Approximately equal numbers of leaves from each of five clones were treated 3 hours in seven test solutions. These solutions comprised various concentrations and combinations of *p*-aminobenzoic acid, indole-3-butyric acid, asparagine, and glucose (table 6). Initial survival was surprisingly uniform for all treatments. Neither rooting nor regeneration was improved by treatment with indole-3-butyric acid, asparagine, or glucose. Response to *p*-aminobenzoic acid concentrations was inconsistent, but in general it did not seem to improve regeneration.

Complete records were kept of the responses of leaves according to their clonal origin, since root cuttings from different clones have different regenerative capacities (unpublished data, and table 2). That such is the case for leaves is apparent from table 7, where the regenerative ability of leaves from six different clones is compared when untreated and when all treatments are combined. These data serve to illustrate the numbers of leaves needed in order to establish with reasonable accuracy the regenerative ability of particular clones. Within clone 93.27, regeneration from actively growing parent plants was particularly noteworthy but was lower when leaves were subsequently tested from inactively growing plants. However, differences in environment between tests may partially account for the observed decrease.

TABLE 3

EFFECT OF TREATING LEAVES FOR 20 HOURS IN 500 MG./L. KNO_3. TOTALS ARE FROM EIGHT CLONES

TREATMENT	No. LEAVES	PERCENTAGE		
		Survival 10 days	Rooted 20 days	Regenerated 70 days
KNO_3	166	51	24	6
Water (control)	176	64	17	8

TABLE 4

EFFECT OF DIPPING LEAF BASES IN INDOLE-3-BUTYRIC ACID-TALC MIXTURES. APPROXIMATELY EQUAL NUMBERS OF LEAVES FROM EACH OF FIVE CLONES WERE TREATED AND RESULTS TOTALED

TREATMENT	CONCENTRATION (P.P.M.)	No. LEAVES	PERCENTAGE			
			Survival 10 days	Rooted 20 days	Rooted 30 days	Regenerated 90 days
Indole-3-butyric	5000	134	37	5	15	2
Indole-3-butyric	2000	92	47	14	17	3
Indole-3-butyric	1000	113	45	12	16	4
Talc (control)		121	55	7	12	1

Forty leaves of *T. latilobum* were tested in the same manner as those of *T. kok-saghyz*. At the end of 10 days, twenty-two leaves survived. Within 30 days, one leaf had a shoot but no roots. Within 60 days, two of the leaves had short roots and were potted in manurial compost. Each of the six unrooted leaves which still survived had several calluses, some as large as peas. A week after potting, one of the rooted leaves produced a shoot which later developed into a vigorous plant.

Discussion

In isolated leaves of *Taraxacum kok-saghyz*, shoots arise from leaf-borne roots (type III, HAGEMANN's classification). The base of the leaf cutting first forms an inconspicuous callus which proceeds to

TABLE 5

EFFECT OF 3-HOUR TREATMENT WITH NAPHTHALENEACETIC ACID, AND WITH A COMBINATION OF ASPARAGINE, GLYCINE, AND *p*-AMINOBENZOIC ACID. TOTALS ARE FROM FOUR CLONES

TREATMENT	CONCENTRATION (MG./L.)	NO. LEAVES	PERCENTAGE			
			Survival 10 days	Rooted 20 days	Rooted 30 days	Regenerated 90 days
Naphthaleneacetic	50	116	59	27	33	3
Asparagine	250					
Glycine	3	128	77	33	39	7
p-Aminobenzoic	1					
Water (control)		121	62	22	30	8

TABLE 6

EFFECT OF TREATING APPROXIMATELY EQUAL NUMBERS OF LEAVES FROM EACH OF FIVE CLONES FOR 3 HOURS IN SOLUTIONS OF *p*-AMINOBENZOIC ACID, INDOLE-3-BUTYRIC ACID, AND COMBINATIONS OF THESE WITH ASPARAGINE AND GLUCOSE

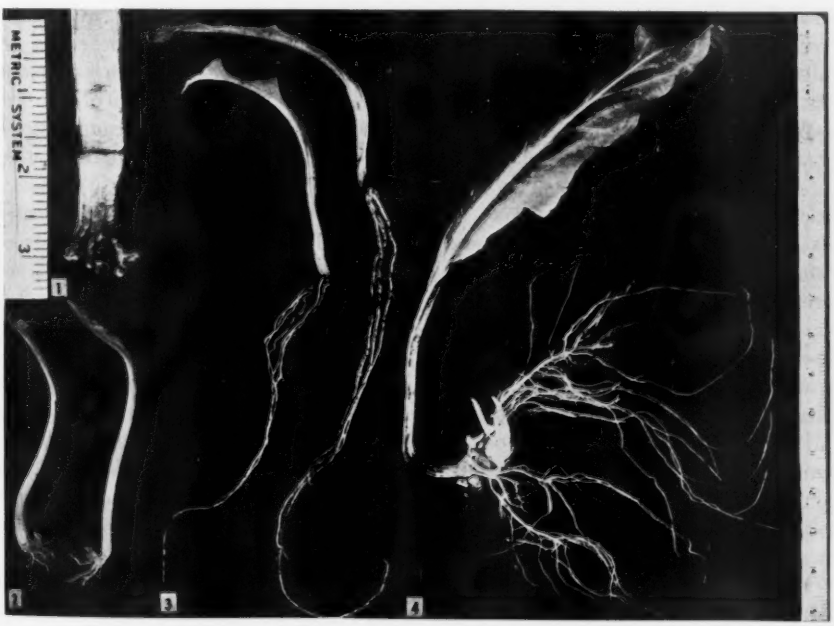
TREATMENT	CONCENTRATION (MG./L.)	NO. LEAVES	PERCENTAGE			
			Survival 10 days	Rooted 20 days	Rooted 30 days	Regenerated 90 days
p-Aminobenzoic	0.1	87	55	15	15	4
p-Aminobenzoic	1	86	57	14	14	1
p-Aminobenzoic	10	86	57	24	24	11
Indole-3-butyric	50	85	60	18	21	8
p-Aminobenzoic	1					
Asparagine	75	87	62	21	23	4
Indole-3-butyric	50					
p-Aminobenzoic	1					
Asparagine	75	85	57	26	27	8
Indole-3-butyric	50					
Glucose	25,000					
p-Aminobenzoic	0.1					
Asparagine	75	87	67	29	23	5
Indole-3-butyric	50					
Water (control)		85	58	28	29	6

TABLE 7

COMPARISON OF REGENERATIVE ABILITY OF LEAVES FROM SIX CLONES WHEN UNTREATED AND WHEN ALL TREATMENTS ARE COMBINED

Clone	No. leaves	Percentage			
		Survival 10 days	Rooted 20 days	Rooted 30 days	Regenerated 90 days
93.27* (Un.)†	44	61	32	34	27
(All)	322	63	26	30	21
93.42 (Un.)	21	57	33	33	0
(All)	334	52	11	18	2
93.24 (Un.)	63	65	19	32	2
(All)	535	61	23	29	2
93.31 (Un.)	43	58	23	26	7
(All)	178	66	39	40	7
253.3 (Un.)	9	67	11	11	0
(All)	120	40	3	4	0
93.22 (Un.)	44	48	27	27	5
(All)	226	54	25	25	1
93.27‡	70	41	7	11	6

* Leaves from actively growing plants.
† Un., untreated; All, all treatments combined.
‡ Leaves from plants in inactive state of growth.



FIGS. 1–4.—Fig. 1, leaf cutting showing calluses at ends of vascular strands. Figs. 2, 3, roots arising from calluses. Fig. 4, developing shoot (parent leaf thickened). Magnification of fig. 1 twice that of figs. 2–4.

develop most noticeably about the severed ends of the vascular strands (fig. 1). From these callus swellings one to several roots arise (fig. 2) which elongate (fig. 3) and frequently become much-branched. Shoots arise from the callus swellings at the apex of the root (fig. 4); exceptionally shoots arise on the root below this point.

Those leaves which survive the first 10 days have callused successfully. With favorable environmental conditions, mortality during the next 10 days is low. During this time the greater part of the root initiation occurs. Thereafter, the amount of root initiation taking place is so limited (table 7) as to make it impractical to retain callused, unrooted leaves any longer. Bits of crown tissue which may remain attached to the leaf base are not essential for regeneration, since leaves with the basal portion removed also regenerate. This ability evidently decreases toward the leaf tip, since the distal portions of leaves were incapable of regeneration.

In a very few instances shoots may arise from unrooted callus swellings, but these have low viability. Concurrent development of root and shoot may take place before the leaves are potted on the twentieth day, but most shoots appear later. Parent leaves which persist become thickened and curled and can still be distinguished when the rosette of the new plant has attained considerable size. Death of the parent leaf is not prejudicial to shoot production if the root system remaining is well established. In fact, the upper portion of the roots may become considerably thickened before shoots appear.

More than one shoot may arise from a rooted leaf cutting. This becomes most evident upon death of a parent leaf, when the separated callus swellings continue to regenerate independently. As many as four regenerated plants have been observed to originate from a single leaf cutting.

Leaves from actively growing plants vary in their capacity to regenerate, depending on their clonal origin. Within a particular clone the state of maturity of the parent plant has little effect on the regenerative capacity of the leaves, provided it is in an active state of growth. Leaves from mature plants of the same clone have a higher or lower regenerative capacity, depending on whether the plants are growing vigorously or not. Moreover, the outer senescent and inner juvenile leaves of a whorl do not regenerate so well as do those in between. Actively growing, physiologically mature leaves seemingly possess factors facilitating regeneration to a greater degree than do leaves which are inactively growing, juvenile, or senescent; and clones therefore differ from one another in the amounts of such factors each is potentially able to produce. None of the substances tested stimulated regeneration or helped to correct deficiencies of these factors.

The foregoing experiments demonstrate the rather limited success to be expected in the propagation of *T. koksaghyz* by means of leaf cuttings. Such practice is warranted only under special circumstances, such as the establishment of polyploid clones that might be compared with the diploid clones from which they were derived. This would avoid the difficulties inherent in diploid-polyploid comparisons between heterogeneous seedling populations. Two tetraploid plants have been obtained by the successful regeneration of leaves from colchicine-treated root cuttings of one clone, providing evidence that such leaves are capable of being propagated.

Summary

1. Factors considered essential in the propagation of leaves of *Taraxacum kok-saghyz* Rod. are: (*a*) removal under water, (*b*) moderate humidity and a temperature of approximately 68°F. in the cutting-frame, and (*c*) potting with the rooted leaf base just below the soil line.

2. Under the conditions of the experiments, treatments with indole-3-butyric acid, naphthaleneacetic acid, glucose, asparagine, glycine, *p*-aminobenzoic acid, and potassium nitrate, in various concentrations and combinations, failed to improve regeneration.

3. Leaf regeneration of *T. kok-saghyz* was by means of shoots arising from leaf-borne roots.

4. Clones differed in their ability to regenerate. The observed variation ranged from 0% to 27%. The condition of growth at the time of testing, rather than the age of the parent plant, seemed to determine variation within a clone.

5. One out of forty leaves of *T. latilobum* DC. regenerated.

Acknowledgment is made of photographs by Dr. MILDRED K. NOBLES of material from Dr. WRAY M. BOWDEN. The assistance of Miss MAY E. LIVINGSTONE is greatly appreciated.

BOTANY AND PLANT PATHOLOGY
SCIENCE SERVICE
DEPARTMENT OF AGRICULTURE
OTTAWA, ONTARIO

LITERATURE CITED

1. BOWDEN, WRAY M., Unpublished data. 1943.
2. HAGEMANN, A., Untersuchungen an Blattstecklingen. Gartenbauwissenschaft **6**:69–195. 1932.
3. MYNBAYEV, K., On the vegetative propagation of kok-saghyz. Soviet Plant Industry Record No. 1: 60-62. 1940.
4. NAYLOR, E., The proliferation of dandelions from roots. Bull. Torr. Bot. Club **68**:351–358. 1941.
5. NAVASHIN, M. S., and GERASSIMOVA, HELEN, Production of polyploid plants from leaves treated with colchicine. C. R. (Doklady) Acad. Sci. U.R.S.S. **24**:948–950. 1939.
6. STINGL, G., Über regenerative Neubildungen an isolierten Blättern phanerogamer Pflanzen. Flora **99**:178–193. 1909.
7. SWINGLE, CHARLES F., Regeneration and vegetative propagation. Bot. Rev. **6**:301–355. 1940.
8. THIMANN, K. V., and POUTASSE, E. F., Factors affecting root formation of *Phaseolus vulgaris*. Plant Physiol. **16**:585–598. 1941.

EFFECTS OF SOAKING WITH INDOLEBUTYRIC ACID ON ROOT DEVELOPMENT AND SURVIVAL OF TREE SEEDLINGS

T. E. MAKI AND HUBERT MARSHALL[1]

Introduction

The last decade has witnessed a phenomenal increase in our knowledge of growth-regulating substances and in our ability to use them to regulate plant behavior. These substances are used today for several commercial purposes, including the rooting of cuttings of woody species heretofore difficult or impossible to root by conventional methods. The widespread use of growth regulators on cuttings naturally has awakened interest in stimulating the production of new roots on transplant stock. Such a need is felt acutely in forest planting, since the nature of the undertaking makes it uneconomical to transplant seedlings to the field with balled and relatively intact root systems. In handling bare-rooted stock, a large portion of the root system is lost in lifting from the nursery bed, or is pruned off to facilitate rapid planting in the field; in consequence, death of seedlings may result from critically reduced water absorption. If growth-regulator treatments could be perfected which would stimulate rapid production of new roots immediately after planting, considerable loss in forest plantations might be avoided.

Studies of root formation on cuttings have shown that growth regulators stimulate the initiation of root primordia, which subsequently elongate under the influence of other factors. Other studies have shown that growth regulators, in concentrations which initiate root primordia, are likely to inhibit the growth of both shoots and roots. The problem, then, becomes one of devising a schedule of treatments which will bring about the initiation of primordia on the old roots but which will not inhibit their subsequent elongation. Several investigators have reported stimulated root development on transplants, but, for the most part, they have given few or no data on subsequent survival. The study here reported was designed to clarify conflicting reports in the literature and to determine whether the root stimulation frequently reported actually can be translated into increased survival.

Review of literature

Several investigators have studied the effects of vitamins on woody plants. BENSEND (2) grew jack-pine seedlings in sand irrigated with a balanced nutrient solution to which thiamin or niacin was added at periodic intervals. After 80 days no differences in size or weight were found, and analysis of the tissue showed that controls contained as much thiamin as treated seedlings. Similarly, PARKER *et al.* (7) found that young Valencia-orange trees planted in soil with bare or balled roots and with adequate water and mineral elements failed to respond to either thiamin or niacin added to the irrigation water.

On the other hand, SWARTLEY and CHADWICK (12) dusted groups of ten rooted cuttings of *Lonicera fragrantissima* with Transplantone no. 1 (a commercial talc preparation of growth regulators and vitamins in undisclosed proportions), and with talc dusts of nico-

[1] Senior Forester and Research Assistant, respectively, Northeastern Forest Experiment Station, Philadelphia, Pa., in co-operation with the University of Pennsylvania.

tinic acid 1–1000 and thiourea 1–20,000, before planting in beds in a slat-house or in pots in a coldframe. Two months later all treatments (especially nicotinic acid) in the slat-house had improved top growth, but no response was noted in the coldframe. In a second experiment, lots of ten seedlings of *Malus sieboldi arborescens* were root-dusted with talc mixtures of nicotinic acid 1–500 and 1–1000, indolebutyric acid 1–5000, and Transplantone no. 1. Indolebutyric acid and Transplantone produced clusters of new roots but nicotinic acid was ineffective. Lastly, lots of twenty-five to seventy-five seedlings of *Juniperus virginiana* and *Picea glauca* were watered with Transplantone, either once or three times, the latter treatment being superior. On *Juniperus*, both number of new roots and survival were increased, but *Picea* failed to respond.

Several reports have appeared on the use of growth regulators on coniferous stock. PLANK (**8**) soaked groups of ten slash-pine seedlings for 24 hours in aqueous solutions of indolebutyric acid ranging between 10 and 80 mg. per liter. One year after planting, root development showed stimulation from the lower concentrations. In a more elaborate experiment, FOWELLS (**4**) grew groups of twenty ponderosa-pine seedlings in nutrient tanks or pots of soil after soaking the roots for 24 hours in 5, 25, or 50 mg. of indoleacetic acid or thiamin per liter of water. After 3 months in the tanks, seedlings soaked in 5 mg./l. indoleacetic acid produced significantly longer roots than did controls. Seedlings planted in soil and treated with 50 mg./l. indoleacetic acid produced significantly more secondary roots than did seedlings treated in any other manner. No other differences were found, and FOWELLS concludes that the results were not encouraging. A similar conclusion was reached by ZACH *et al.* (**14**) after treating a number of coniferous species with indolebutyric acid and planting them in a nursery and on an adverse site in the field.

Somewhat more work has been reported on hardwoods. LEFEVRE (**6**) applied talc dusts of indolebutyric and indoleacetic acids in concentrations of 1-10,000 and 1-100,000 to the roots and grafts of grapevines, with subsequent improvements in root development and quality of callus. CHADWICK (**3**) soaked the roots of 3-year-old plants of *Viburnum dilatatum* and *Cotoneaster divaricata* for 18 hours in aqueous solutions containing 20, 50, and 100 mg./l. of indoleacetic, indolebutyric, or phenylacetic acid. After 69 days, *Viburnum* treated with 20 mg. of indolebutyric acid had developed seven times more new roots than controls. *Cotoneaster*, after the same length of time, had responded most favorably to the same substance at 50 mg./l. A similar response was obtained by adding 10 or 20 mg. of the acids to the soil in 5-inch pots. Applying the growth regulators by dipping the roots into emulsified paraffin containing the acids was not successful.

TILFORD (**13**) lifted seedlings of American elm, hard maple, black oak, pin oak, black walnut, white pine, Norway spruce, and American arborvitae in January and soaked the roots for 48 hours in growth regulators at 25 mg./l. The seedlings were potted and remained in the greenhouse until April, when all except white pine and Norway spruce showed definite root stimulation. Indolebutyric acid produced the greatest response. In a second experiment, American-elm and red-oak saplings were root-pruned, treated, and planted in the field. Dipping in, or watering with, growth

regulators, or smearing the cut ends of the roots with lanolin mixtures, failed to induce response; but soaking the roots for 48 hours in 40 mg./l. indolebutyric acid (the better substance) or indoleacetic acid more than doubled the number of new roots by the end of the first growing season.

At the Rubber Research Institute of Malaya (1), budded stumps of rubber trees were lifted from the nursery, root-pruned about 18 inches below the collar, and soaked for 16 or 24 hours in a dilute solution of a commercial growth regulator covering only the lower 1½ inches of the taproot. Substantial increases were obtained in the number of stumps taking root and in the total root production.

SMITH and ROMBERG (10) devised a method of treating pecan transplants with growth regulators which they regard as superior to either soaking or localized lanolin treatment. The method consists of soaking toothpicks in 95% alcohol in which indolebutyric acid has been dissolved in such concentration as to allow the picks to absorb 4 mg. of the acid. From one to several picks are then inserted into holes bored transversely through the larger roots. The acid diffuses into the surrounding tissues, where it locally stimulates root formation. Using this method, treated 5- and 7-year-old pecan trees developed, by the end of the first growing season, about three times as many new roots and four times as much new root length as untreated controls (9). In later experiments (11), the dry weight of new root growth on 5-year-old pecan trees was increased in one test from 8.2 gm. on controls to 15.6 gm. on treated plants, and in another test from 4.8 gm. to 10.0 gm. Equally striking results were obtained with large trees having trunk circumferences of approximately 14 inches. Although none of the tests was carried out with the primary object of obtaining survival data, wherever mortality occurred controls suffered more than treated trees. GOSSARD (5) made an additional test of the technique of SMITH and ROMBERG. He found that treated pecan trees produced significantly more root growth than controls but significant differences in survival and shoot growth were not obtained.

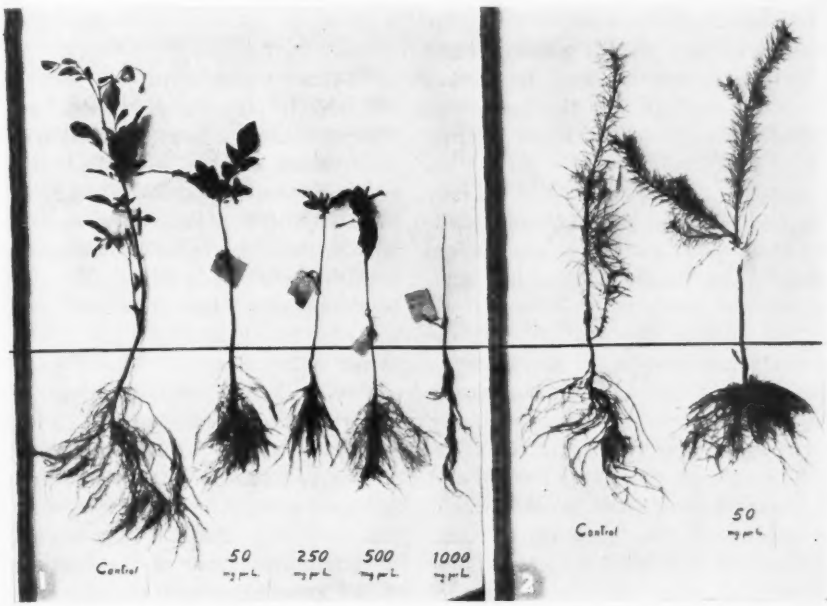
Investigation

PRELIMINARY EXPERIMENTS, 1942

Although the toothpick technique of SMITH and ROMBERG may be of value in treating orchard or horticultural stock, it obviously would be impracticable with the small-sized trees commonly used in forest planting. Of the other treatments, dusting and soaking the roots seemed to offer promise, particularly in view of TILFORD's work (13). Accordingly, an exploratory study[2] was carried out in 1942 to test the effects of growth regulators on a wide variety of plant materials. During late spring and early summer, seedlings or small plants of twenty-six species of evergreen and deciduous trees and shrubs were treated by soaking the roots for 24 hours in aqueous solutions of indolebutyric acid in concentrations ranging from 0 to 1000 mg./l. Twenty plants per treatment were used. Immediately after treatment, the stock was planted in a plowed field according to a statistically acceptable design. At the end of the summer the plants were dug and determinations made of total weights, root quality and volume, and amount of new top growth. In general,

[2] This study was conducted in co-operation with the Hormone Project of the Plant Industry Station at the Beltsville Research Center. Assistance of Drs. WM. S. STEWART and JOHN W. MITCHELL in planning the tests is gratefully acknowledged.

concentrations of 250 mg./l. and higher resulted in poorer root and shoot growth and considerable increase in mortality. Concentrations below 250 mg./l. produced variable results. With nursery-grown stock with adequate survival, for example, significant increases in root inhibited shoot growth in proportion to concentration, and the higher levels unquestionably were harmful, in some instances causing leaves to fall prematurely, even though stem and roots remained alive (fig. 1). Among species suffering heavy mortality in the con-



FIGS. 1, 2.—Fig. 1, white-ash seedlings, 1 year old when treated, showing effect of indolebutyric acid gradient on shoot and root development 2 months after treatment. Soaking roots in the acid caused inhibition of shoots, even in lower concentrations. On an absolute basis, treated seedlings had smaller root volumes; on a basis relative to shoot growth, the reverse was frequently true. Fig. 2, jack-pine seedling on right treated by soaking roots for 24 hours in indolebutyric acid at 50 mg./l. This treatment was generally not beneficial, but occasional seedlings responded with greatly stimulated root development.

volumes were found on loblolly (*Pinus taeda* L.) and shortleaf (*Pinus echinata* Mill.) pines, but no beneficial responses occurred with red (*Pinus resinosa* Ait.), white (*Pinus strobus* L.), and pitch (*Pinus rigida* Mill.) pines, nor with white ash (*Fraxinus americana* L.), tuliptree (*Liriodendron tulipifera* L.), and paper birch (*Betula papyrifera* Marsh.). In many species, root soaking trols, only a few treatments brought about increases in survival. Altogether, these results were not particularly encouraging. However, many examples of root stimulation on individual seedlings were observed (fig. 2). While the marked responses of these seedlings were not great enough or frequent enough to result in significant increases for their treatment groups as a whole, they did

provide hope that root growth (and probably survival) could be increased if the factors causing the individual responses could be determined.

Preliminary experiments, 1943

The following spring an effort was made to determine with greater precision the effects of growth regulators on tuliptree and loblolly pine. It was felt that if definite prescriptions could be worked out for a few species, the knowledge gained would greatly facilitate adaptation of such treatments to other species. Accordingly, groups of sixty loblolly pine and tuliptree seedlings were root-soaked for either 4 or 22 hours in aqueous solutions of naphthaleneacetic acid, naphthalene acetamide, indolebutyric acid, or all four combinations of these three substances at 30, 90, or 270 mg./l. This design departed from the earlier study primarily in the reduced number of species, the lower concentrations, the addition of a short soaking period, and the comparison of both single growth regulators and mixtures in factorial combinations. Detailed studies of root development were not made, but survival tallies showed consistent increases in mortality resulting from almost all treatments. Indolebutyric acid was less toxic than the other substances and at the lowest concentration was not harmful.

Tuliptree was used both years and failed to benefit in either root growth or survival. Loblolly pine responded in 1942 with stimulated root development but showed no benefits in 1943. These tests gave little promise that soaking the roots in solutions of the compounds would substantially reduce mortality of forest planting stock. However, other tests in 1943 with growth regulators applied to hardwood stock in common storage showed again that under the proper circumstances root growth can be greatly stimulated. Accordingly, a small experiment was begun in May, 1944, to study in greater detail the effects of indolebutyric acid on the root development of one pine and one hardwood species.

Main experiments, 1944

Material and methods.—One-year-old loblolly pine was selected as a test species because it had been used in both preliminary studies and had shown favorable root response in 1942. One-year-old eastern red oak (*Quercus borealis maxima*) was chosen as the other species because closely related species of the black-oak group had responded well in the 1942 study and in Tilford's (13) earlier experiments.

The stock was carefully graded to a uniform size, root-pruned, and tied into groups of ten seedlings for determination of root volumes by water displacement in a graduated cylinder. This procedure was adopted so that the volumes could be determined again at the conclusion of the experiment and the increase in volume used as a measure of treatment differences.

In general, the method of treatment was the same as that employed in the earlier work. In addition to untreated controls, the treatments consisted of soaking the roots for 24 hours in solutions containing 0 (tap water only), 2.5, 5, 10, 20, 40, 80, or 160 mg. of indolebutyric acid per liter of water. A surplus lot of loblolly pine was utilized by soaking the roots of similar groups for 6 hours in all concentrations except 5, 20, and 80 mg./l.

The planting was done May 5, 1944, in four well-drained beds (6 × 6 feet) of a 50-50 mixture of sand and partly

rotted sawdust. Each species was planted in two blocks, the treatments being randomly arranged in rows of twenty seedlings each. Thus, each row consisted of two groups of ten seedlings on which root volumes had been determined. Spacing within the row was $3\frac{1}{2}$ inches and spacing between rows was 4 inches for loblolly and $5\frac{1}{2}$ inches for oak. A complete nutrient solution at the rate of $\frac{1}{2}$ oz. of salts per square foot was applied several times during the summer.

In October, 1944, the seedlings were removed from the beds by washing the sand and sawdust from the roots with a garden hose. The surviving seedlings of the original groups of ten were tied together and the root volumes again determined by water displacement.

RESULTS.—The data on survival and root growth for red oak are presented in table 1. A moderate trend is shown toward increased survival through the lower concentrations and up to 20 mg./l., with a decided drop in survival above that concentration. The only seedlings surviving significantly better than controls were those soaked in 5 mg./l. In contrast, survival of seedlings treated with the highest two concentrations was significantly poorer than controls.

Root growth was stimulated by even the lowest concentration of acid, and a marked increase in volume was produced by all concentrations over 20 mg./l. The uniformly greater root volume resulting from all treatments between 2.5 and 20 mg./l. was not statistically significant, although from the uniformity of the data in this concentration range it appears that the differences are real. The increase in volume from treatments above 20 mg./l. is highly significant.

If there had been only a slight increase in root growth with increasing concentration of indolebutyric acid, the reliability of the data representing mean growth might be questioned, inasmuch as the figures given are differences between mean root volumes of all seedlings as planted and mean root volumes of surviving seedlings at the end of the growing season. If only small trees had died among treated groups, these groups would, without benefit of treatment effects, tend to show greater mean growth than controls. However, the stimulation of root growth was readily apparent (fig. 3) and the range of original seedling sizes was not sufficient, under any condition of differential mortality, to cause such large treatment differences. And, finally, if differential death of small seedlings in treated groups had caused the increased mean root volume, one would expect the treatment at 5 mg./l., with no mortality, to have a smaller mean growth than the treatments at 2.5, 10, or 20 mg. Such was not the case, and therefore the conclusion seems fully

TABLE 1

SURVIVAL AND ROOT GROWTH OF GROUPS OF FORTY EASTERN RED-OAK SEEDLINGS ROOT-SOAKED 24 HOURS IN AQUEOUS SOLUTIONS OF INDOLEBUTYRIC ACID. TREATED MAY 4, 1944; RECORD OBTAINED OCTOBER, 1944

Concentration of indolebutyric acid (mg./l.)	No. surviving*	Original mean root volume (ml.)	Final mean root volume (ml.)	Mean root growth (ml.)†
Untreated..	33	7.50	8.48	0.98
0.0.....	35	6.88	7.43	0.55
2.5.....	33	7.12	9.54	2.42
5.0.....	40	7.38	10.12	2.74
10.0.....	37	7.12	9.46	2.34
20.0.....	36	7.62	10.00	2.38
40.0.....	30	7.62	12.17	4.55
80.0.....	20	7.50	15.25	7.75
160.0.....	9	7.12	13.33	6.21

* Least significant difference at 5% level, 6.9; at 1% level, 9.4.

† Least significant difference at 5% level, 2.50; at 1% level, 3.37.

warranted that the treatment differences are real.

The results with loblolly pine are presented in table 2. The trends observed in the data for oak are not apparent in the results for pine. Mortality increased with higher concentrations of indolebutyric acid but the effect was not regular. Root growth was stimulated by most concentrations, but again there was no consistent correlation with treatment.

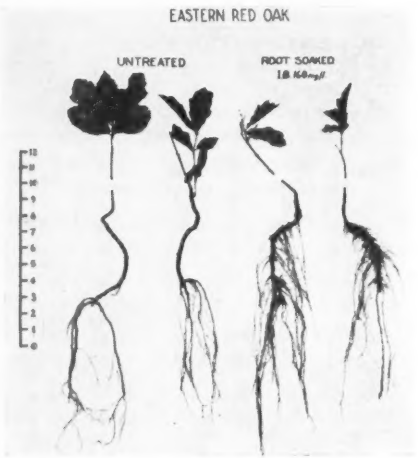


Fig. 3.—Root stimulation on 1-year-old eastern red-oak root soaked 24 hours in 160 mg. indolebutyric acid per liter of water. Survival of seedlings at this concentration greatly reduced. Photographed 5 months after planting in sand-sawdust bed.

The increase in mortality with the stronger treatments, however, indicates that for this species the series of concentrations used extended well into the toxic range.

Discussion

Soaking the roots of red oak in solutions of indolebutyric acid stimulated root growth over the entire range in concentration. This agrees with the results of others who have worked with hardwoods, as well as with some of the results obtained with hardwoods in preliminary studies at this station. The failure of tuliptree to respond with increased root growth in the preliminary studies is perhaps an exception among hardwoods.

TABLE 2

SURVIVAL AND ROOT GROWTH OF GROUPS OF FORTY LOBLOLLY-PINE SEEDLINGS ROOT-SOAKED 6 OR 24 HOURS IN AQUEOUS SOLUTIONS OF INDOLEBUTYRIC ACID. TREATED MAY 4, 1944; RECORD OBTAINED OCTOBER, 1944

Concentration of indolebutyric acid (mg./l.)	No. surviving	Original mean root volume (ml.)	Final mean root volume (ml.)	Mean root growth (ml.)
24 hours:				
Untreated..	17	0.91	3.50	2.59
0.0.....	26	0.91	3.54	2.63
2.5.....	19	0.85	3.32	2.47
5.0.....	25	0.85	3.90	3.05
10.0.....	20	0.85	4.10	3.25
20.0.....	11	0.85	5.95	5.10
40.0.....	15	0.84	3.93	3.09
80.0.....	14	0.88	3.87	2.99
160.0.....	7	0.86	4.21	3.35
6 hours:				
Untreated..	30	0.85	3.15	2.30
0.0.....	24	0.85	5.04	4.19
2.5.....	19	0.86	3.68	2.82
10.0.....	28	0.90	4.14	3.24
40.0.....	21	0.90	5.12	4.22
160.0.....	10	0.88	4.40	3.52

The survival results obtained with red oak are of importance because as yet there have been no wholly satisfactory reports on survival of forest seedlings after treatment with growth regulators. Most studies have not considered this factor; some have been too small to be of much value. ROMBERG and SMITH (9, 11) found that survival was higher in treated groups, but they did not use the soaking technique. GOSSARD (5), however, was unable to find significant differences while using the same technique and species. The 1943 preliminary study at

this station showed definite increases in mortality of tuliptree following treatment with growth regulators. This effect has now been duplicated on red oak for all concentrations above 20 mg./l. Below 20 mg. there appears to be some hope of increasing survival. Soaking treatments with indolebutyric acid apparently stimulate root development of some hardwoods to a limited extent without increasing mortality, but substantial increases in development are obtained only at the expense of reduced survival.

The root response of loblolly pine was likewise in tenor with most published results. Of those working with *Pinus* or *Picea*, only PLANK (**8**) reported truly positive results. His work was not extensive, however, and has not been followed with confirming reports. FOWELLS (**4**) reported several isolated examples of stimulation but felt his results were not encouraging. In the 1942 preliminary tests, loblolly and shortleaf pine responded with increased root growth, but in later trials with loblolly these results could not be duplicated. Different lots of the same species evidently exhibit a wide range in response to growth-regulating chemicals. On the whole, it seems likely that under certain conditions soaking with growth regulators will stimulate root development on conifers, but those conditions have not yet been defined.

The mortality response of loblolly pine confirmed both previous studies at this station, as well as the work of ZACH *et al.* (**14**). No other work on the mortality of pines has appeared. On the basis of present evidence, however, it seems improbable that survival of pines can be increased by soaking the roots with growth regulators.

Summary

1. Seedlings of eastern red oak and loblolly pine were treated immediately before planting by soaking the roots for 24 hours (in addition to a 6-hour series for loblolly pine) in aqueous solutions of indolebutyric acid ranging in concentration from 2.5 to 160 mg. per liter. Root volumes were measured before planting and again when the seedlings were lifted at the end of the first growing season.

2. Consistent increases in root volume of red oak followed soaking in all concentrations of indolebutyric acid, and statistically significant increases occurred with concentrations of 40 mg./l. or more.

3. A barely significant increase in survival followed treatment of red oak with 5 mg./l. On the other hand, a marked and highly significant increase in mortality followed treatment with 80 mg./l. or more. The largest increases in root volume of red oak were associated with the largest increases in mortality.

4. Treatments generally increased the root volume of loblolly pine, although there was no consistent relationship between root volume and concentration.

5. A marked increase in mortality of loblolly pine followed treatment with concentrations above 20 mg./l.

SOUTHERN FOREST EXPERIMENT STATION
GULFPORT, MISSISSIPPI

LITERATURE CITED

1. ANONYMOUS. Root stimulation by hormones. Agr. Jour. Ceylon **93**:56–59. 1939.
2. BENSEND, DWIGHT W., Effect of thiamin and niacin on growth of jack-pine seedlings. Jour. Forest. **40**:883–884. 1942.
3. CHADWICK, L. C., Root development of *Viburnum dilatatum* and *Cotoneaster divaricata* with the use of growth substances. Arborist's News **2** (10):3–4. 1937.
4. FOWELLS, H. A., The effect of certain growth

substances on root-pruned ponderosa pine seedlings. Jour. Forest. 41:685–686. 1943.

5. GOSSARD, ATHERTON C., Root and shoot production by young pecan trees treated with indolebutyric acid at the time of transplanting. Proc. Amer. Soc. Hort. Sci. 41:161–166. 1942.
6. LEFEVRE, J., Results observed after phytohormone treatment of grapevine grafts. Compt. Rend. Acad. d'Agr. de France 25:629–632. 1939.
7. PARKER, E. R., TURRELL, F. M., and BONNER, JAMES, Effects of organic matter and certain growth substances on the development of young orange trees in the orchard. Proc. Amer. Soc. Hort. Sci. 38:49–58. 1941.
8. PLANK, DONALD K., Root response of slash pine seedlings to indolebutyric acid. Jour. Forest. 37:497–498. 1939.
9. ROMBERG, L. D., and SMITH, C. L., Effects of indole-3-butyric acid in the rooting of transplanted pecan trees. Proc. Amer. Soc. Hort. Sci. 36:161–170. 1939.
10. SMITH, C. L., and ROMBERG, L. D., A method for the treatment of cuttings and roots of the pecan with root-inducing chemicals. Plant Physiol. 14:177–178. 1939.
11. ———, and ———, Use of indolebutyric acid as an aid in transplanting pecan trees. Unpublished manuscript. 1941.
12. SWARTLEY, JOHN C., and CHADWICK, L. C., Effects of synthetic growth substances on cuttings, seeds, and transplants. Bimonthly Bull. Ohio Agr. Exp. Sta. 27:125–144. 1942.
13. TILFORD, PAUL E., Effect of some synthetic growth substances on root development of transplanted trees. Proc. 14th Nat. Shade Tree Conf. Pp. 51–59. 1938.
14. ZACH, LAWRENCE W., BAUER, DON, and GOODYEAR, HAL, Practical application of plant hormones in forest-tree propagation. Jour. Forest. 41:214. 1943.

EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE GROWTH OF GRASS PLANTS

JOHN W. MITCHELL[1] AND PAUL C. MARTH[2]

Introduction

The use of 2,4-dichlorophenoxyacetic acid as a selective herbicide for eradicating such turf weeds as dandelion and plantain has recently been suggested (4, 5). Most of the experimental results on this method of control indicate that application of the acid or salts (sodium or ammonium) of the acid, at the rate of about $1\frac{1}{2}$ pounds per acre, does not noticeably affect the appearance of well-established bluegrass or some other varieties of grass commonly used in lawns. Attention for the most part has been directed toward a study of the weed-killing properties of 2,4-dichlorophenoxyacetic acid, rather than toward its effects on the germination and growth of young grass seedlings or older grass plants (1, 2, 3).

The present experiments, in which the treatments were applied to the soil immediately following planting, were undertaken for the purpose of determining the effect of the acid on (*a*) the emergence of grass seedlings of different species, (*b*) the subsequent growth of the various grasses, and (*c*) grass plants well established when sprayed.

[1] Physiologist, [2]Physiologist; Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, Beltsville, Maryland.

Experimental data

SEEDLING EMERGENCE

METHODS.—Weighed amounts of seeds of redtop (*Agrostis alba*), creeping red fescue (*Festuca rubra*), and Kentucky bluegrass (*Poa pratensis*) were planted in composted soil contained in clay pots 12 inches in diameter and 6 inches deep, with bottom drainage. On October 20,

1944, forty pots were sown with each species of grass seed at the rate of 0.5 gm. per pot, a total of 120 pots being used.

After the seeds had been covered lightly with soil, the surface was sprayed evenly with a measured amount of an aqueous mixture of the acid, applied with a hand sprayer of one-pint capacity operating at a pressure of 80 pounds. The aqueous mixture contained 0.1% of the acid and 0.5% Carbowax 1500 applied at rates equivalent to 2, 4, 6, and 8 gallons per thousand square feet—or $\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{4}$, and 3 pounds of the acid per acre. Similar pots of soil were planted but left unsprayed as controls. The treatments were then arranged in randomized blocks on a greenhouse bench so that two pots of the same variety and treatment constituted a plot. There were four replications of each plot. Temperature was maintained at 65°–75° F. throughout the experiment.

TABLE 1

AVERAGE PERCENTAGE EMERGENCE OF GRASS SEEDLINGS 1 WEEK AFTER SPRAYING SOIL SURFACE WITH AQUEOUS MIXTURE CONTAINING 0.1% 2,4-DICHLOROPHENOXYACETIC ACID AND 0.5% CARBOWAX 1500; SPRAY APPLIED IMMEDIATELY AFTER SEEDS WERE PLANTED. AVERAGE NUMBER OF SEEDLINGS THAT EMERGED IN UNSPRAYED PLOTS DESIGNATED AS 100%

Rate of treatment (equivalent lb. per acre)	Redtop	Fescue	Bluegrass
0	100	100	100
$\frac{3}{4}$	72	110	114
$1\frac{1}{2}$	17	102	80
$2\frac{1}{4}$	17	90	71
3	5	83	68

The number of grass seedlings that appeared above the surface of the soil 7 days after planting was recorded and designated as emergence. Although some seedlings may have emerged after this time, no subsequent counts were made owing to the difficulty involved in obtaining an accurate estimation of their number.

RESULTS.—Spray mixture at a rate equivalent to $2\frac{1}{4}$ and 3 pounds of acid per acre reduced the emergence of all three varieties of grass seedlings as compared with the number that appeared above the surface of the unsprayed soil (table 1). The emergence of plants of redtop was much more drastically affected by the chemical than was that of either fescue or bluegrass. In the case of fescue, a greater percentage of seedlings appeared above the surface of soil sprayed with small amounts of the acid ($\frac{3}{4}$ and $1\frac{1}{2}$ pounds per acre) than emerged from the unsprayed plots. A greater number of bluegrass seedlings also appeared above the surface of soil sprayed with the mixture at a rate equivalent to $\frac{3}{4}$ pounds per acre than emerged from unsprayed soil. This experiment was continued so as to determine subsequent effects of treatment on seedling growth.

SEEDLING GROWTH

METHODS.—After the emergence counts had been taken and the leaves of grass had attained a length of approximately 3 inches, they were cut back uniformly to a height of 2 inches, and this height was maintained by clipping at weekly or bi-weekly intervals. The fresh weights of the clippings were recorded.

At the end of 5 weeks after treatment, all plants in one of the pots in each replicate of each treatment were pulled up, and each of these pots was then reseeded with 0.5 gm. of seed of the respective varieties. The weights of weekly clippings from plants that developed in these reseeded plots were recorded and are summarized separately.

RESULTS.—The seedlings that emerged in the treated soil did not outwardly appear to differ from those in the untreated soil. The weights of clippings from all treated plots of the original seeding of redtop were significantly less than those from the unsprayed ones for a period of 1 month (table 2). During this period, however, grass growing in soil sprayed at a rate equivalent to $\frac{3}{4}$ pound of the acid per acre multiplied, apparently by means of stolons or rhizomes, and during the last 10 weeks of the experiment it was as productive as was that growing in untreated soil. At the end of 9 weeks, the weights of clippings of redtop growing in soil sprayed at the rate of $1\frac{1}{2}$ pounds per acre were not appreciably less than those of the unsprayed plots. The production of clippings by redtop in plots sprayed at the rates of $2\frac{1}{4}$ and 3 pounds per acre was appreciably less than that of the unsprayed plots during the entire period of 14 weeks, at which time the experiment was discontinued.

Fescue growing in soil sprayed at the rate of $\frac{3}{4}$ pound of the acid per acre became increasingly productive, and after the fifth week the weights of clippings obtained were not appreciably less than those from the unsprayed plots. Plots of fescue sprayed at rates of $1\frac{1}{2}$ pounds or

TABLE 2

AVERAGE FRESH WEIGHT IN GRAMS OF GRASS CLIPPINGS COLLECTED AT WEEKLY INTERVALS AFTER PLANTS HAD GERMINATED IN SOIL SPRAYED WITH AQUEOUS MIXTURE CONTAINING 0.1% 2,4-DICHLOROPHENOXYACETIC ACID AND 0.5% CARBOWAX 1500

Rate of treatment (equivalent lb. per acre)	Weeks after treatment												Total
	3	4	5	6	7	8	9	10	11	12	13	14	
	Redtop												
0	3.7	9.0	8.6	4.6	5.4	5.5	6.6	3.8	2.1	2.4	2.1	5.2	59.0
$\frac{3}{4}$	2.0	5.7	10.1	4.2	6.1	5.9	5.6	4.0	1.9	2.7	2.0	6.4	56.6
$1\frac{1}{2}$	0.1	0.3	1.8	1.3	2.5	3.3	4.0	2.4	2.0	2.8	2.7	7.6	30.8
$2\frac{1}{4}$	0.0	0.1	0.6	0.6	0.9	1.3	1.9	1.4	1.4	2.5	1.5	4.4	16.6
3	0.0	0.0	0.2	0.2	0.4	0.6	0.7	0.8	0.9	1.2	1.0	4.2	10.2
	Fescue												
0	3.0	2.3	3.4	2.4	3.0	3.1	4.1	3.4	2.4	2.5	2.6	5.1	37.3
$\frac{3}{4}$	1.3	1.0	2.2	1.7	1.4	2.6	3.2	2.6	1.7	2.1	2.0	4.4	26.2
$1\frac{1}{2}$	0.6	0.6	1.6	0.8	1.5	1.7	1.9	1.6	1.4	1.5	1.9	3.1	18.2
$2\frac{1}{4}$	0.4	0.4	1.0	0.6	1.0	1.1	1.2	1.3	1.1	1.7	1.6	3.7	15.1
3	0.5	0.4	0.9	0.7	1.1	1.5	1.5	1.2	1.1	1.3	1.7	3.4	15.3
	Bluegrass												
0	1.6	4.2	6.0	3.2	3.5	3.6	4.4	3.4	2.3	2.8	2.8	5.8	43.6
$\frac{3}{4}$	0.1	0.7	3.2	1.9	3.3	3.5	3.8	3.0	2.0	2.6	2.3	4.3	30.7
$1\frac{1}{2}$	0.0	0.3	2.2	1.6	2.6	2.7	2.6	2.0	1.6	2.5	2.1	4.0	24.2
$2\frac{1}{4}$	0.0	0.2	1.9	1.2	2.1	2.5	2.1	1.7	1.4	1.9	1.7	3.8	20.5
3	0.0	0.1	1.1	0.9	1.5	1.8	1.7	1.3	1.2	1.4	1.6	3.5	16.1

more per acre failed to equal the unsprayed plots in productivity.

The productivity of bluegrass plots was significantly reduced on the basis of the clippings obtained, but within 7 weeks after seeding plants growing in soil sprayed at the rate of $\frac{3}{4}$ pound of acid per acre attained a production of clippings not significantly less than that of the unsprayed plots. Eleven weeks were required, however, before plots treated at the rates of $1\frac{1}{2}$ and $2\frac{1}{4}$ pounds per acre were as productive as were the unsprayed ones. Bluegrass growing in soil sprayed at the rate of 3 pounds per acre failed to recover during the 14-week period.

Five weeks after application of the spray treatments there were no apparent or measurable effects, either on the emergence of seedlings in reseeded plots or on the subsequent growth of the grass, indicating that the acid had been either inactivated or leached from the soil during this time (table 3).

TABLE 3

AVERAGE FRESH WEIGHT IN GRAMS OF CLIPPINGS FROM GREENHOUSE GRASS PLOTS RESEEDED 5 WEEKS AFTER SOIL SURFACE WAS SPRAYED WITH AQUEOUS MIXTURE CONTAINING 0.1% 2,4-DICHLOROPHENOXYACETIC ACID AND 0.5% CARBOWAX. PLANTS OF FIRST SEEDING REMOVED BEFORE RESEEDING

Rate of treatment (equivalent lb. per acre)	Weeks after seeding								Total
	3	4	5	6	7	8	9	11	
	Redtop								
0	1.0	4.3	2.3	2.3	3.0	3.3	7.7	12.1	36.0
$\frac{3}{4}$	1.7	4.8	3.2	2.1	3.0	3.4	8.0	11.5	37.7
$1\frac{1}{2}$	2.4	5.5	3.8	3.3	3.2	2.9	9.7	16.1	46.9
$2\frac{1}{4}$	2.0	5.1	3.6	3.0	3.0	2.5	6.7	10.7	36.6
3	2.0	4.6	3.1	2.6	2.4	2.9	9.8	15.3	42.7
	Fescue								
0	1.4	1.0	0.5	0.6	0.8	0.8	2.8	10.5	18.4
$\frac{3}{4}$	1.7	1.1	0.6	0.5	0.8	0.9	3.4	9.1	18.1
$1\frac{1}{2}$	2.2	1.0	0.9	0.6	0.9	0.7	2.6	7.5	16.4
$2\frac{1}{4}$	2.1	1.2	0.8	0.7	0.7	1.0	4.7	9.0	20.3
3	2.4	1.4	0.9	0.7	0.9	1.1	4.1	9.6	21.1
	Bluegrass								
0	0.4	1.2	1.0	1.1	1.6	2.0	3.7	6.6	17.6
$\frac{3}{4}$	0.3	1.1	1.3	1.3	2.0	2.5	5.0	7.0	20.5
$1\frac{1}{2}$	0.3	1.4	1.7	1.6	2.2	2.4	4.7	8.1	22.4
$2\frac{1}{4}$	0.5	1.5	1.4	1.5	1.6	1.9	6.2	8.7	23.3
3	0.4	1.5	1.5	1.7	1.8	2.3	4.8	7.6	21.6

GROWTH OF BENTGRASS

METHODS.—On October 23, 1944, nine clumps of stolons of the Washington strain of creeping bentgrass (*Agrostis*

palustris), each consisting of six stolons, selected for size and uniformity, were planted 1–2 inches apart in each of forty clay pots containing composted soil. The pots were similar to those already described. On November 11, after the grass had become well established, the pots were separated into five groups of eight pots each. The grass in each of four groups was then sprayed with an aqueous mixture containing 0.1% of the acid and 0.5% Carbowax 1500 at a rate equivalent to $\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{4}$, or 3 pounds per acre, respectively. The eight remaining pots were left untreated. After treatment, the pots were arranged in randomized blocks on a greenhouse bench with four replications of each treatment. Throughout the experiment the grass was clipped back to a height of 2 inches at approximately weekly intervals and the fresh weights of the clippings recorded.

TABLE 4

AVERAGE FRESH WEIGHT IN GRAMS OF BENTGRASS CLIPPINGS PRODUCED PER POT IN GREENHOUSE, COLLECTED AT MONTHLY INTERVALS, AFTER SPRAYING WITH AQUEOUS MIXTURE CONTAINING 0.1% 2,4-DICHLOROPHENOXYACETIC ACID AND 0.5% CARBOWAX 1500

RATE OF TREATMENT (EQUIVALENT LB. PER ACRE)	MONTHS AFTER TREATMENT				TOTAL
	1	2	3	4	
0	18.4	16.2	14.1	32.7	81.4
$\frac{3}{4}$	14.4	13.1	16.9	32.7	77.1
$1\frac{1}{2}$	9.1	8.2	11.3	30.5	59.1
$2\frac{1}{4}$	8.7	7.8	8.8	29.3	54.6
3	6.5	5.9	7.3	26.4	46.1

RESULTS.—The total weight of clippings produced by bentgrass was reduced significantly below that of the unsprayed plants as the result of treatment at all rates of the spray mixture used (table 4). Within 1 month following treatment the sprayed plants developed enlarged regions at the nodes of the rhizomes, and the leaf blades were a less intense green and somewhat narrower than were those on the unsprayed plants. The sprayed plants gradually improved in appearance, however, and at the end of the experiment there were no apparent differences between them and the controls.

The growth of bentgrass was not significantly affected by the spray mixture during the first week following treatment. All treatments depressed growth during the second, third, and fourth weeks, so that the total weight of clippings during the first month was significantly less than in the case of unsprayed plants (table 4). Plots sprayed at the rate of $\frac{3}{4}$ pound per acre recovered, and following the first month after treatment they produced an amount of clippings equal to or slightly greater than that from the unsprayed plots. Cuttings from plots sprayed at the rates of $1\frac{1}{2}$, $2\frac{1}{4}$, and 3 pounds per acre weighed 6.7, 10.4, and 19.3% less, respectively, than those cut from unsprayed plots during the fourth month after treatment.

ESTABLISHED TURF

METHODS.—On March 26, 1945, an area of turf having a uniformly mixed stand of the three species of grass—redtop, creeping red fescue, and Kentucky bluegrass—was selected and marked off into thirty parallel strips 2 feet wide and 25 feet long. Treatments consisted in spraying the individual strips with a water mixture containing 0.1% of the acid and 0.5% Carbowax 1500 at the same equivalent rates as were used in the previous greenhouse experiments—0, $\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{4}$, and 3 pounds per acre. The various treatments were assigned at random to the plots so as to make six randomized blocks.

A hand-pressure knapsack sprayer was

used in applying the treatment, and plyboard shields were held along the sides of each area as it was being sprayed to avoid drift of the mixture. All weeds on the entire area were removed by hand at the beginning of the experiment to eliminate competition between the weeds and grass and to make it possible to observe the direct effect of the acid on growth of the grass.

At intervals of several weeks, a strip 18 inches in width was mowed lengthwise through the middle of each plot, the mower being equipped with a grass catcher. The weights of the clippings were recorded immediately after cutting. The entire area was mowed evenly after the clipping samples had been taken.

RESULTS.—Grass in the treated plots showed no apparent signs of injury, but weights of clippings taken 18 days following treatment indicated that the growth of plants treated with even the lowest spray concentration (equivalent to $\frac{3}{4}$ pound per acre) was significantly less than that of the untreated plants (table 5). During the second month after treatment, clippings from plots sprayed with an equivalent of $\frac{3}{4}$ pound per acre weighed approximately 14.6% more than did those from the unsprayed plots; but following this period there was no consistent difference between these two treatments. The total weight of clippings obtained from plots sprayed with an equivalent of $\frac{3}{4}$ pound per acre over a 6-month period was not appreciably less than of those from the unsprayed plots.

During the 6-month period following treatment, the total weight of clippings from plots sprayed at the rate of $1\frac{1}{2}$ pounds per acre was 11.6% less than that produced by the unsprayed plots. The growth of grass on plots sprayed with greater amounts of the acid ($2\frac{1}{4}$ and 3 pounds per acre) was not consistently different throughout the experiment from growth on those sprayed with $1\frac{1}{2}$ pounds per acre. In all cases the treatment caused an initial depression in the rate of growth. This depression gradually disappeared, and at the end of approximately 2 months there was no significant difference between the weights of clippings from the treated and those from the untreated plots.

During the latter part of the 6-month period following treatment, weed seedlings grew in the control plots but not in any of the treated ones. In September, 6 months after treatment, the grass and weed leaves in samples of clippings from

TABLE 5

AVERAGE FRESH WEIGHT IN GRAMS OF CLIPPINGS FROM GRASS TURF COMPOSED OF KENTUCKY BLUEGRASS, CREEPING RED FESCUE, AND REDTOP, COMPARED WITH THAT FOR AREAS OF EQUAL SIZE SPRAYED WITH AQUEOUS MIXTURE CONTAINING 0.1% 2,4-DICHLOROPHENOXYACETIC ACID AND 0.5% CARBOWAX

RATE OF TREATMENT (EQUIVALENT LB. PER ACRE)	DAYS AFTER TREATMENT									TOTAL
	18	37	52	78	88	102	126	151	188	
0	510	445	458	424	396	482	423	877	269	4284
$\frac{3}{4}$	381	506	525	475	328	457	353	842	274	4141
$1\frac{1}{2}$	349	420	478	370	311	417	351	823	268	3787
$2\frac{1}{4}$	339	390	507	470	305	425	348	777	275	3836
3	344	352	479	432	340	465	373	779	267	3831

the unsprayed plots were separated. The weights of the weed clippings in six replicated unsprayed plots were 32, 34, 32, 30, 31, and 30% of the total weight of clippings from each plot, respectively, while the weight of weed leaves in clippings from all other treatments was estimated to be only a fraction of 1% of the total weight of the samples, since only a few weed leaves were found in some of the samples of grass taken from the treated plots, while others were completely free of weeds.

Discussion

In previous experiments (**3**, **5**), satisfactory stands of Kentucky bluegrass seedlings were established in lawn areas by broadcasting the seeds just prior to spraying with a water mixture of 2,4-dichlorophenoxyacetic acid in the fall. These earlier results indicated that at least some of the seeds sown on a well-established lawn at the time of spraying may germinate and grow. The number which become established, however, may depend somewhat on protection from the spray mixture that is afforded to the seeds and soil by leaves of grass and weeds present on the area at the time of treatment. Results of the present experiments indicate that it is somewhat more difficult to establish seedling grass plants in bare soil that has been sprayed than it is in unsprayed soil.

The detrimental effects of the spray mixtures on soil were not persistent under greenhouse conditions, however, since seeds of the three species of grass germinated readily and became established when planted in the soil 5 weeks after its surface had been sprayed. In experiments reported by others (**2**), seeds of several crop plants sown in soil 58 days after it was treated with 2,4-dichlorophenoxyacetic acid germinated readily and the plants grew vigorously. Also, seeds of Kentucky bluegrass became established in a lawn area that had been treated with the acid 54 days earlier.

Effort should be made to avoid spraying the acid mixture directly onto fallow soil in which grass seedlings are to be established soon after treatment. On the basis of the present experiments it would appear that a satisfactory stand of new grass can be more readily obtained if the seeds are planted 4–6 weeks after treatment, rather than if planted at the time the spray treatments are applied.

The species of bentgrass used responded somewhat differently from bluegrass, fescue, and redtop when the leaves were sprayed with the mixture, in that it showed outward signs of the detrimental effect of the acid and its growth was markedly suppressed, while other grasses (bluegrass, fescue, and redtop) did not change appreciably in appearance as the result of treatment, and their growth was only temporarily inhibited. Of course, the results obtained under the conditions of these experiments might vary, depending upon the strains of grasses used, the season of the year, and the weather conditions under which the plants were grown. It should also be mentioned that the effect on the growth of the grass owing to the elimination of weed competition through the herbicidal effects of the treatment has not been studied, since all weeds were removed from both treated and untreated areas at the beginning of the experiments.

The observed depression of growth of grasses as a result of application of the acid to their leaves would appear to be of little practical consequence, in so far as

grass plants not used as forage are concerned. Further experiments are necessary to determine the significance of the effect of the acid on the productivity of pastures or range areas where the chemical might be used for herbicidal purposes. The results of these experiments, however, indicate that the depressing effects on relatively mature grass are not lasting; and where weeds in pastures or range areas seriously reduce the amount of grass produced, it would appear feasible to eliminate them by the use of 2,4-dichlorophenoxyacetic acid.

Summary

1. Potted soil in which Kentucky bluegrass, redtop, and creeping red fescue grass seeds had been planted was sprayed with a water mixture containing 0.1% 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500 at rates equivalent to $\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{4}$, and 3 pounds of the acid per acre. The treatments reduced the number of redtop seedlings that appeared by 28, 83, 83, and 95%, respectively. A slightly greater number of fescue seedlings emerged from the soil sprayed at rates equivalent to $\frac{3}{4}$ and $1\frac{1}{2}$ pounds of acid per acre, while the heavier applications reduced emergence by 10 and 17%, in comparison with the unsprayed soil. Fourteen per cent more bluegrass seedlings appeared in the soil sprayed at a rate equivalent to $\frac{3}{4}$ of a pound per acre than emerged in unsprayed soil. The heavier applications reduced the emergence of bluegrass by as much as 32% below that of untreated soil.

2. Grass seedlings that became established in soil sprayed with various amounts of the water mixture of the acid were allowed to grow under greenhouse conditions for a period of 14 weeks, and their rate of growth was measured each week by means of weights of leaf clippings. Weights of clippings from plants of all three species in treated soil were less than those from the same species grown in untreated soil. Each of the three species, however, showed a tendency to recover from the effects of the sprays. Judging by weights of clippings, the grass seedlings in soil treated with $\frac{3}{4}$ and $1\frac{1}{2}$ pounds per acre had recovered after 5–11 weeks, but at the heavier rates ($2\frac{1}{4}$ and 3 pounds) per acre the grass was still retarded in growth after 14 weeks.

3. The detrimental effects of the acid in the soil were only temporary, since redtop, fescue, and bluegrass seeds germinated and the seedlings became established in treated soil as readily as in untreated soil, when the seeds were planted 5 weeks after the soil was sprayed.

4. The growth of well-established creeping bentgrass was depressed by spraying with the water mixture at rates equivalent to $\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{4}$, and 3 pounds of the acid per acre. Plants sprayed at rates equivalent to $\frac{3}{4}$ and $1\frac{1}{2}$ pounds per acre recovered during a period of 3–4 months following treatment.

5. The appearance of turf growing out-of-doors and made up of Kentucky bluegrass, creeping red fescue, and redtop was unchanged after application as a water spray at rates equivalent to $\frac{3}{4}$, $1\frac{1}{2}$, $2\frac{1}{4}$, and 3 pounds per acre. Weight of clippings was reduced, however, during a period of 1–2 months after treatment, but after this interval there was no difference between the growth of sprayed and unsprayed plots.

Bureau of Plant Industry, Soils and Agricultural Engineering
Beltsville, Maryland

LITERATURE CITED

1. BLACKMAN, G. E., Comparison of certain plant growth substances with other selective herbicides. Nature 155:506–508. 1945.
2. HAMNER, C. L., and TUKEY, H. B., Selective herbicidal action of midsummer and fall applications of 2,4-dichlorophenoxyacetic acid. BOT. GAZ. 106:232–245. 1944.
3. MARTH, P. C., and MITCHELL, JOHN W., 2,4-Dichlorophenoxyacetic acid as a differential herbicide. BOT. GAZ. 106:224–232. 1944.
4. MITCHELL, JOHN W., and HAMNER, C. L., Polyethylene glycols as carriers for growth-regulating substances. BOT. GAZ. 105:474–483. 1944.
5. MITCHELL, JOHN W., DAVIS, F. F., and MARTH, P. C., Clover and weed control in turf with plant-growth regulators. Golfdom: October, 1944.

EFFECT OF GROWTH-REGULATING SUBSTANCES ON THE DEVELOPMENT OF APPLE SCALD

HAROLD A. SCHOMER AND PAUL C. MARTH[1]

Introduction

During an investigation by C. L. HAMNER and the senior writer concerning the effects of growth-regulating substances on the keeping quality of apples in cold storage, it was observed that the treated fruits developed considerably less scald than the untreated ones during the storage season of 1943–44. The growth substances had been applied to the fruits after harvest and just prior to placing in cold storage. The effects of the treatments were significant enough to warrant further investigation in the storage season of 1944–45. The results reported here for these two seasons are considered preliminary, but they show a positive effect of treatment and are in sufficient agreement to warrant a report at this time.

Methods

Apple varieties used in the investigation for the 1943–44 season were Grimes Golden, Stayman Winesap, and York Imperial. In the 1944–45 season the first two varieties were used, and Arkansas (Mammoth Black Twig), a variety especially susceptible to scald, was substituted for York Imperial.

In treating the fruits, duplicate lots of approximately 1 bushel selected at random from the entire bulk of material were used for each treatment. The treatments were applied by two methods: (*a*) as a dip, which was a lanolin emulsion containing varying amounts of the different growth substances; and (*b*) as an aerosol, applied by the use of a liquefied gas containing the chemical in solution (5).

The lanolin emulsion for the dip treatments was prepared in lots of 10 liters each and contained 4 gm. of pure lanolin per liter. In preparing the emulsion, 7.5 gm. of stearic acid was melted in 100 ml. hot water and 2.7 gm. of trimethanolamine added while stirring. Forty grams of previously melted lanolin was then added and the mixture stirred vigorously until cool. Water was added to the thick emulsion, slowly at first, with constant stirring, until the 10 liters were obtained. In preparing the final mixtures for dipping, the growth substances were first dissolved in 95% ethyl alcohol in the proportion of 1:10, and the necessary quantities of the alcoholic solution were added to the prepared lanolin emulsion to give the desired concentrations. Each growth

[1] Associate Physiologist and Physiologist; Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, Beltsville, Maryland.

substance (or a mixture of them) was applied in one or all of three concentrations, the total amount of the substances present being 10, 100, and 500 p.p.m., respectively. Following treatment, the fruits were allowed to dry and were placed in boxes in 31° F. storage for a period of 4–5 months.

The growth substances used were α-naphthaleneacetic acid, β-indolebutyric acid, and a mixture in equal parts of these two acids, plus β-naphthoxyacetic acid and α-naphthalene acetamide.

For the aerosols, dimethyl ether (b.p. −27° C.) was used as the volatile solvent. In each instance the aerosol mixture consisted of 1% of the growth substance, 10% of cyclohexanone as solvent, and 89% of dimethyl ether as the volatile agent. The fruits to be treated were spread on a drying screen and the aerosol was directed on them from above and below to insure complete coverage.

In classifying the scalded fruits, three arbitrary degrees of severity of scald were designated as slight, medium, or severe. Slight scald denoted discoloration of a mild intensity, or that a very small area was affected on the individual fruits, the disorder being considered so slight as to be unobjectionable. Medium scald denoted moderate discoloration, such that the fruits would probably be discriminated against on the market. Severe scald indicated fruits that were decidedly objectionable, the scalded area including 50% or more of the fruit surface. Figure 1 illustrates three lots of fruits sorted into four grades (sound, and the three degrees of scald).

Results

Arkansas (Mammoth Black Twig) experiments, 1944–45

During the 1944–45 season, the most striking results were obtained with the Arkansas variety. The fruit was harvested October 19, 1944, and placed at 31° F. for 5 days until treated on October 24. On April 18, 1945, the fruits were removed from cold storage and placed at 70° F. for 7 days to allow time for maximum scald development. Final examination for scald was made on April 25. Considerably less scald was found in all lots treated with the compounds than in the check lots (table 1). On the basis of scald development, the best treatment consisted of the lanolin emulsion containing 100 p.p.m. α-naphthaleneacetic acid. Fruits receiving this treatment were 33.1% sound and 33.7% slightly scalded, as compared with the check (untreated) fruits in which only 9.1% were sound and 15.6% slightly scalded. Medium and severe scald in these same lots were, respectively, 22.3 and 9.0% in the treated fruits and 19.9 and 49.9% in the untreated. Little difference was found in the percentages of sound and slightly scalded fruits among the different treated lots. The lanolin emulsion without growth regulators also showed a tendency to reduce scald development, as indicated in table 1 and figure 1.

From these results it may be seen that the intensity of scald development, as well as the total number of fruits affected by the disorder, was reduced by treatment with growth-regulating substances.

Stayman Winesap experiments 1943–44 and 1944–45

Although a marked reduction in scald development was obtained on Stayman Winesap apples in both storage seasons, the degree of scald reduction differed considerably. The variance in response may have been due to differences in maturity of the fruits when picked, to different sources of the material, or to delay in treating the fruits after harvest (1–3).

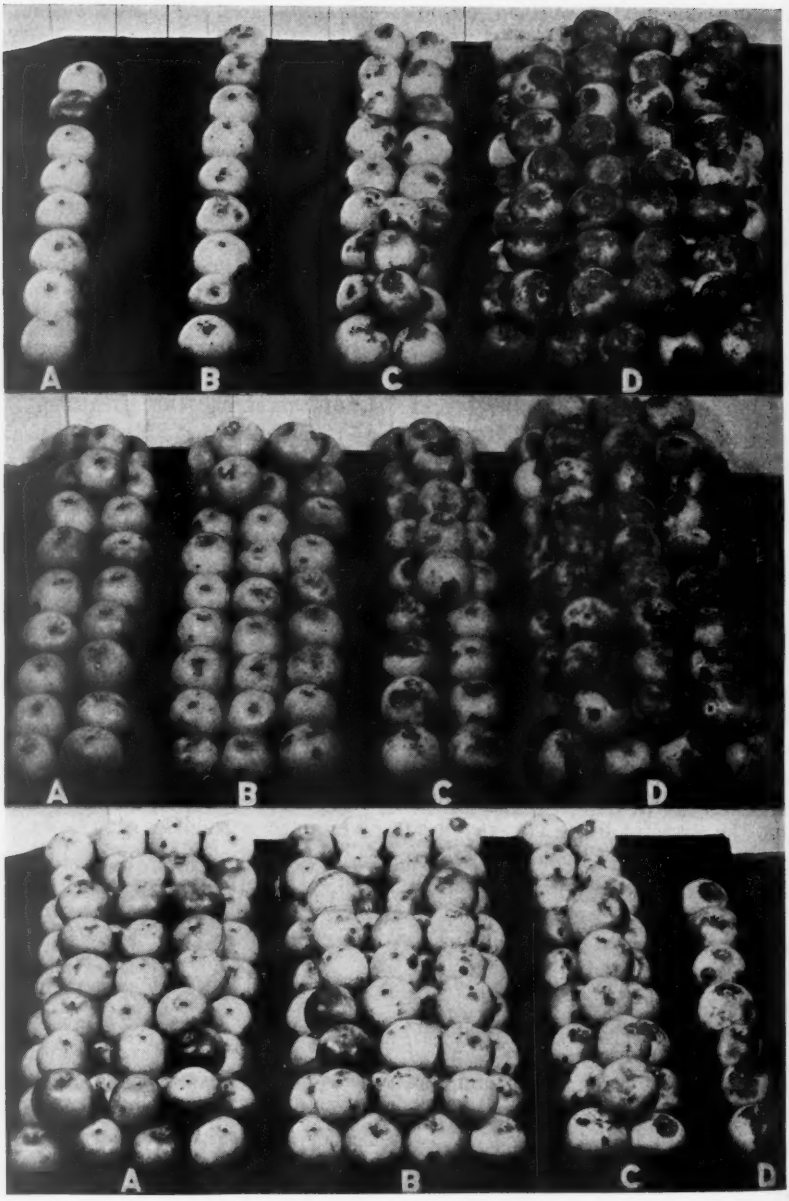


FIG. 1.—Scald development on Arkansas apples at end of storage period on April 25, 1945. *A*, sound; *B*, slightly scalded; *C*, moderately scalded; *D*, severely scalded. Upper row, untreated fruits. Middle row, fruits dipped in lanolin emulsion. Lower row, fruits dipped in lanolin emulsion containing 500 p.p.m. α-naphthaleneacetic acid.

Fruit for the 1943–44 storage season was picked on October 10, 1943, from an orchard near Mt. Jackson, Virginia. It was held for 5 days at 31° F. prior to treatment on October 15. Following treatment, the apples were held in 31° F. storage until April 21, when they were removed to 70° F. and held for 7 days before final examination on April 28. It was on the treated fruits of this lot that the first observations were made of striking scald reduction due to the compounds. In these preliminary investigations three concentrations (10, 100, and 500 p.p.m.) in lanolin emulsion of naphthaleneacetic acid, of indolebutyric acid, and of the mixture of four growth substances were employed. All treatments resulted in less scald than occurred in the untreated control. Little variation in the percentage of scalded fruits, due to varying concentrations of the growth regulators, was found. The average percentages of scalded fruits of all lots treated with naphthaleneacetic acid, indolebutyric acid, and the mixture were 21.5, 25.6, and 17.2, respectively; in contrast, comparable untreated fruits were 62.3 scalded. The percentages of sound fruits for the same treatments were 70.7, 67.5, and 75.4, respectively, whereas the control fruits were 31.1% sound.

In the 1944–45 season the fruit was harvested on the Plant Industry Station grounds at Beltsville, Maryland, on October 8, 1944, and stored at 31° F. for 16 days prior to treatment on October 24. Final examination of the fruits was made April 6, after they had been held for 1 week at 70° F. to allow time for maximum scald development. The greatest scald reduction was obtained with naphthaleneacetic acid at 500 p.p.m. in lanolin emulsion (table 2). The effectiveness of this substance declined with decrease in concentration. The mixture of the four growth substances in lanolin emulsion was somewhat less effective than the same concentration (100 p.p.m.) of naphthaleneacetic acid used alone, while the mixture used as aerosol produced no reduction in scald. Consider-

TABLE 1

EFFECT OF GROWTH-REGULATING SUBSTANCES ON SCALD DEVELOPMENT ON ARKANSAS (MAMMOTH BLACK TWIG) APPLES, 1944–45. FRUITS TREATED AND STORED AT 31° F. ON OCTOBER 24, 1944; REMOVED FROM COLD STORAGE ON APRIL 18, 1945, AND PLACED AT 70° F. FOR 7 DAYS PRIOR TO MAKING FINAL SCALD RECORDS

Treatment	Sound fruit (%)	Percentage scald			Total decay (%)
		Slight	Medium	Severe	
Naphthaleneacetic acid (in lanolin emulsion)					
10 p.p.m.	24.6	37.7	17.5	11.6	8.2
100 p.p.m.	33.1	33.7	22.3	9.0	2.0
500 p.p.m.	30.3	33.3	16.2	16.8	3.3
1% aerosol	21.3	34.3	29.7	10.5	4.2
Mixture*					
100 p.p.m. in lanolin emulsion.	31.3	34.3	17.5	14.2	2.7
1% aerosol	27.5	35.4	19.9	15.2	2.0
Lanolin emulsion only	16.0	21.5	19.5	40.4	2.6
Check (untreated)	9.5	15.6	19.9	49.9	5.1

* Equal parts of naphthaleneacetic acid, indolebutyric acid, naphthoxyacetic acid, and naphthalene acetamide, in lanolin emulsion or in aerosol.

able injury was produced by the aerosol application, with subsequent decay of fruits, a number of which otherwise might have been classified as free from scald. Some scald reduction was produced by the lanolin emulsion alone, but incidence of decay was very high from this treatment, owing chiefly to one of the duplicate lots in which 51% of the fruits showed some decay. No explanation can be given for the high percentage in this lot.

check and a second check lot dipped in a lanolin emulsion completed this test. The apples were picked September 15, treated and placed in storage at 31° F. on September 16, and examined for scald on January 11, after 1 week at 70° F.

Although the percentages of sound (not scalded) fruits did not vary greatly between the treated and untreated lots (8.6 and 9.3%, respectively), the intensity of the scald was reduced by treatment, so that the average percentage of

TABLE 2

EFFECT OF GROWTH-REGULATING SUBSTANCES ON SCALD DEVELOPMENT ON STAYMAN WINESAP APPLES, 1944–45. FRUIT HARVESTED OCTOBER 8, 1944; STORED AT 31° F. FOR 16 DAYS; TREATED AND RE-STORED AT 31° F. OCTOBER 24; REMOVED FROM STORAGE MARCH 31, 1945; PLACED AT 70° F. FOR 7 DAYS AND EXAMINED FOR FINAL SCALD DEVELOPMENT APRIL 6

TREATMENT	SOUND FRUIT (%)	PERCENTAGE SCALD			TOTAL DECAY (%)
		Slight	Medium	Severe	
Naphthaleneacetic acid (in lanolin emulsion)					
10 p.p.m.	29.5	12.6	23.7	24.5	9.7
100 p.p.m.	35.6	11.9	20.6	14.9	17.0
500 p.p.m.	44.1	14.2	20.4	7.8	13.5
1% aerosol	29.7	13.9	21.7	13.7	21.0†
Mixture*					
100 p.p.m. in lanolin emulsion.	29.8	16.7	17.2	24.3	12.0
1% aerosol	20.4	14.7	19.6	18.6	26.7†
Lanolin emulsion only	23.1	12.7	16.3	31.9	33.7
Check (untreated)	20.5	19.8	31.0	17.5	11.2

* Equal parts of naphthaleneacetic acid, indolebutyric acid, naphthoxyacetic acid, and naphthalene acetamide, in lanolin emulsion or in aerosol.

† Includes fruits injured by aerosol spray, too much solvent having been applied.

GRIMES GOLDEN AND YORK IMPERIAL EXPERIMENTS, 1943–44 AND 1944–45

Grimes Golden apples that were treated with growth substances had consistently less scald than the untreated checks, although the reduction of scald was not so great as that found in the Arkansas and Stayman Winesap varieties. The only treatments applied to Grimes Golden during the 1943–44 season were three concentrations of the mixture of growth substances. An untreated

severely scalded fruits in the treated lots was 40.5 and in the untreated lots 61.9. Furthermore, the percentages of fruits with slight and medium scald averaged 20.9 for the treated lots and 8.6 for the untreated check. As was found with the other varieties, treatment with lanolin emulsion without growth substances tended to reduce the intensity of scald on Grimes Golden. Similar results were obtained on Grimes Golden during the 1944–45 season, the chief effect of the

treatments being to bring about a reduction in the severity of the scald.

Scald development was light on both the treated and untreated lots of the York Imperial variety for the 1943–44 season, and no attempt was made to classify according to degree of scald. The untreated check lot had the greatest percentage of scalded fruits, 13.1% showing some degree of the disorder. The average percentages of scalded fruits in the treated lots were as follows: naphthaleneacetic acid 9.7, indolebutyric acid 7.1, and the mixture 5.1. Thus, the reduction of scald, although slight, was consistent for all treatments.

The use of lanolin emulsion for application of the growth substances did not seem to affect decay development in the fruits of any variety, the total decay for the lanolin-dipped fruits in all the experiments being 10.0% as compared with 12.0% for the untreated. Injury occurred in some instances where the aerosol vapor was used, probably because an excessive amount of the cyclohexanone solvent was applied during treatment. Pathogens readily invaded these injured areas, with the result that a high percentage of decay occurred.

The lanolin emulsion alone usually effected a slight reduction in scald. Figure 1 illustrates a lot of Arkansas apples dipped in lanolin emulsion in comparison with an untreated lot and with a lot dipped in lanolin emulsion containing 500 p.p.m. naphthaleneacetic acid.

Discussion

Growth-regulating chemicals applied to apples to prevent pre-harvest drop may affect their storage quality indirectly because of the more advanced maturity of later pickings made possible by the sprays. HALLER (4), however, found that α-naphthaleneacetic acid when applied to the trees as a pre-harvest spray had no effect on the firmness of the fruit or on the development of decay, breakdown, or scald during subsequent storage when sprayed were compared with unsprayed apples picked at the same time.

Acceleration of ripening of several kinds of fruits due to growth substances applied after harvest has been reported by MITCHELL and MARTH (6), and the effect of these substances in reducing apple scald may have been associated with their effect on the rate of ripening, since it is known that the incidence of scald is less on mature than on immature fruits (1–3).

In this investigation, less scald occurred on apples treated with growth substances than on the untreated fruit. Also, the various varieties tested responded differently, the most striking results being obtained with Arkansas. This does not necessarily mean that the growth-regulating compounds are more effective on Arkansas; a number of uncontrolled factors, such as varietal susceptibility to scald, maturity of fruit when picked, and delay before treatment, may all have contributed to the results obtained.

All the growth substances employed had a similar effect on scald, no one compound being obviously superior to another. In all probability, more effective chemicals or combinations of them for reducing scald may be found.

Although the application of these growth substances has not decreased scald development to a satisfactory degree, it has given sufficient promise to encourage further investigation of the problem.

Summary

1. Experiments were conducted on apples during the 1943–44 and 1944–45 storage seasons to determine the effect

of growth-regulating substances on development of fruit scald.

2. Two substances, α-naphthaleneacetic acid and β-indolebutyric acid, and a mixture consisting of equal parts of α-naphthaleneacetic acid, α-naphthalene acetamide, β-naphthoxyacetic acid, and β-indolebutyric acid were tested at 10, 100, and 500 p.p.m. concentration in lanolin emulsion (0.4% lanolin) on fruits of Arkansas (Mammoth Black Twig), Stayman Winesap, Grimes Golden, and York Imperial varieties. The fruits were treated after harvest and prior to 31° F. storage by dipping in lanolin emulsion containing the various growth regulators or by using a liquefied-gas aerosol of the substance. The amount of scald development was noted at the end of the storage season of each variety after the fruits had been held for 7 days at 70° F. to allow time for maximum scald development.

3. Treatment resulted in consistently less scald than on untreated fruit. The greatest reduction occurred on the Arkansas variety, where there was an average of 24% more unscalded fruits in the treated than in the untreated lots. Less marked reduction occurred on Stayman Winesap, Grimes Golden, and York Imperial.

4. No appreciable differences were noted between the various compounds or between the concentrations employed. The lanolin-emulsion dip treatments appeared to give slightly better control of scald than did the aerosol treatments.

5. The decrease in scald development was manifest as a reduction both in severity of scald and in total number of fruits affected.

BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING
BELTSVILLE, MARYLAND

LITERATURE CITED

1. BROOKS, CHARLES, COOLEY, J. S., and FISHER, D. F., Apple scald. Jour. Agr. Res. 16:195–217. 1919.
2. ———, Nature and control of apple scald. Jour. Agr. Res. 18:211–240. 1919.
3. ———, Oil wrappers, oils, and waxes in the control of apple scald. Jour. Agr. Res. 26:513–536. 1923.
4. HALLER, MARK H., Effect of preharvest drop sprays on the storage quality of apples. Proc. Amer. Soc. Hort. Sci. 44:207–210. 1943.
5. HAMNER, C. L., and SCHOMER, H. A., Aerosol, a new method of applying growth regulators to plants. Science 99:85. 1944.
6. MITCHELL, JOHN W., and MARTH, PAUL C., Effects of 2,4-dichlorophenoxyacetic acid on the ripening of detached fruit. BOT. GAZ. 106:199–207. 1944.

EMBRYO SAC AND FERTILIZATION IN CYPRIPEDIUM SPECTABILE

B. G. L. SWAMY

Introduction

In 1907, PACE (5) discussed the development of the embryo sac and fertilization in four species of *Cypripedium*—*C. spectabile*, *C. parviflorum*, *C. pubescens*, and *C. candidum*. She claimed that, after the division of the megaspore mother cell, the upper dyad cell promptly degenerates, while the nucleus of the lower cell divides twice to form four nuclei, two lying at each pole of the sac. No further divisions were said to occur; the two nuclei at the micropylar pole organize into two synergids, and the chalazal pair migrates upward to form the egg and a polar nucleus. Subsequently, at the time of fertilization, the nucleus of one of the synergids loosens itself and migrates downward, fusing with the single polar nucleus and the second male gamete to form the primary endosperm nucleus. This mode of development, which began to be known as the *Cypripedium*-type, was generally believed to be correct until RUTGERS (9) put forward some strong objections against it and suggested a reinvestigation.

From a study of PACE's illustrations, RUTGERS offered the following alternative interpretation: After the 4-nucleate stage is reached, the upper of the two daughter nuclei at the micropylar pole divides again, resulting in the formation of three nuclei at this end. Of these, the two sister nuclei of the last division give rise to the two synergids and the lower nucleus constitutes the egg. The two chalazal nuclei remain undivided and move up to function as the polars.

While the objections put forward by RUTGERS against the interpretation of PACE are no doubt justified, his own interpretations give rise to fresh difficulties. There is not a single authentic instance to indicate that both polar nuclei may be derived from the same end of the embryo sac, nor is there any evidence that only one of the two micropylar nuclei (at the 4-nucleate stage) may divide again while the other remains dormant. Three cases of this kind are those of *Garcinia kydia* and *G. treubii* studied by TREUB (13) and of *Moringa oleifera* investigated by RUTGERS himself. Here the embryo sacs are monosporic and the development proceeds normally up to the 4-nucleate stage, but only one of the micropylar nuclei is said to divide further, while the remaining micropylar nucleus and the two chalazal nuclei remain undivided. The resulting embryo sac is thus 5-nucleate. PURI (7, 8) demonstrated the untenability of this hypothesis and showed that the embryo sac is really 8-nucleate and develops normally, the deceptive appearance being caused by an early degeneration of the antipodal cells. The only difference between RUTGERS' interpretation of PACE's figures and his own account of *Moringa*—as well as that of TREUB on *Garcinia*—is that in *Cypripedium* we are dealing with a bisporic embryo sac and in the other two genera with a monosporic one. RUTGERS, however, admits that his interpretation of PACE's figures is "a possible explanation" and not a "decision," which can only be arrived at by a reinvestigation.

PROSINA (6) investigated *C. guitatum* and found that the embryo sac develops according to the *Allium*-type, and also that the primary chalazal nucleus may

either remain undivided or divide only once, so that the chalazal part has only one or two nuclei instead of the normal four. Fertilization is reported as normal. FRANCINI's (2) studies on some species of *Paphiopedilum*, another genus of the Cypripedilinae, show a similar type of development.

In view of these differences and uncertainties, SCHNARF (10–12) and MAHESHWARI (4) suggested a reinvestigation of the species studied by PACE. This seems to have been undertaken by CARLSON (1), but the only paper so far published by her[1] does not contribute much toward the solution of the problem—beyond her statement that in many of her preparations she saw more than four nuclei in the embryo sac.

Material

When the late Professor M. A. SAMPATHKUMARAN returned to India from Chicago about the year 1918, he brought with him some inbedded material of *C. spectabile*, which he very kindly turned over to the writer shortly before his death. From the label on the wrapper I gather that it was given to him by the late Professor C. J. CHAMBERLAIN when he was a student of the latter at the University of Chicago. As *C. spectabile* happens to be one of the species on which PACE based her original observations and interpretations, I gladly undertook to reinvestigate it and record here my appreciation of the opportunity afforded by this material.

Observations

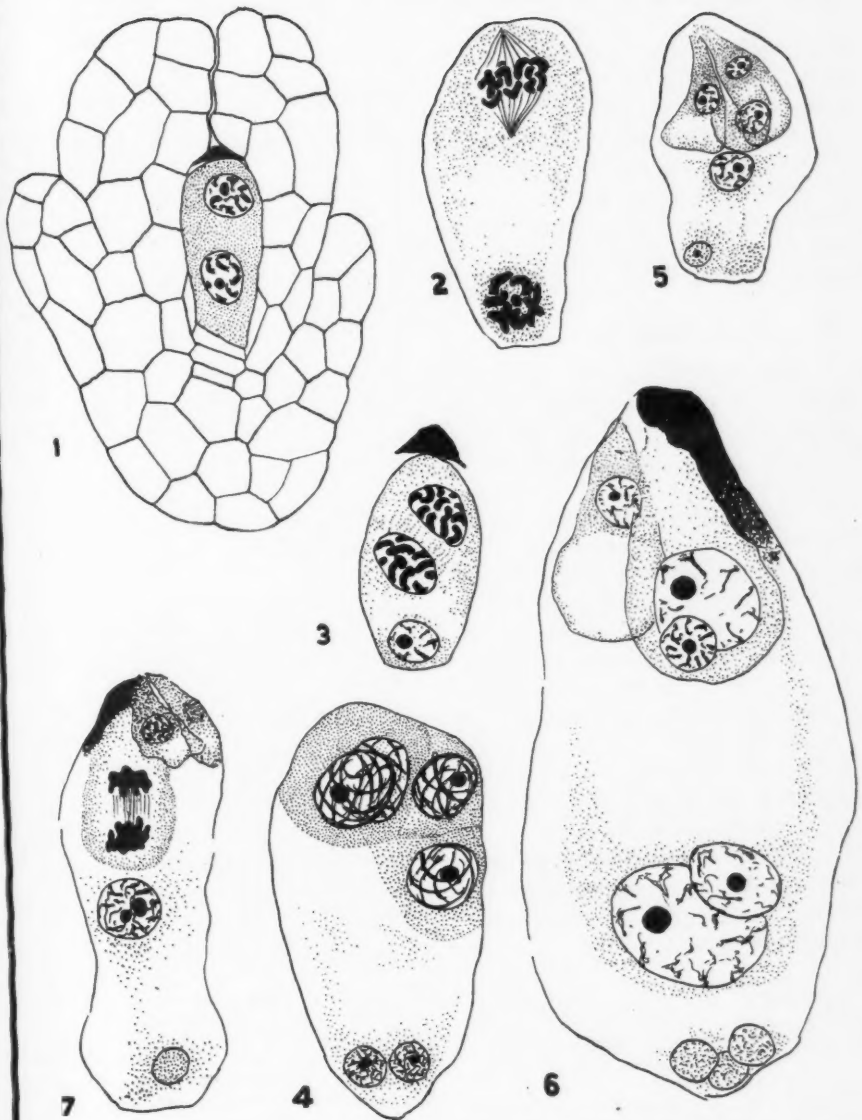
My observations on the structure of the ovule and the development of the embryo sac up to the 2-nucleate stage are in close agreement with those of PACE. The megaspore mother cell on division gives rise to two dyad cells, of which the upper regularly degenerates while the nucleus of the lower divides to form two nuclei unseparated by a wall or membrane (fig. 1). These move apart to opposite poles, and, at the time they embark upon the next division, a large vacuole makes its appearance in the center of the sac (fig. 2). Frequently the primary micropylar nucleus divides first, while the chalazal one remains dormant for some time (fig. 3). Usually the latter divides only once (fig. 4); one of the two daughter nuclei then functions as the lower polar nucleus and the other is to be regarded as the single antipodal. In rare cases one or both nuclei were found to go through the next division, forming three or four nuclei at the chalazal end. In the latter case three antipodals can be seen in the lower end of the embryo sac (fig. 6).

The primary micropylar nucleus regularly undergoes two divisions to form four daughter nuclei. The two spindles are at right angles to each other, and the available evidence indicated that the two synergids on the one hand and the egg and upper polar nucleus on the other are formed from sister nuclei.

The two polar nuclei usually fuse before fertilization (fig. 5). In a few cases both the chalazal nuclei, one being the lower polar nucleus and the other the single antipodal, were found lying in close juxtaposition with the upper polar nucleus.

The pollen tube enters the micropyle and touches the embryo sac close to the point of insertion of the egg apparatus. Here it pierces the embryo-sac membrane, demolishing one of the synergids lying in its way and discharging the two

[1] Since this paper by SWAMY was accepted for publication, an article on *Cypripedium* has appeared by CARLSON (BOT. GAZ. 107:107–114. 1945).



FIGS. 1–7.—Fig. 1, 2-nucleate embryo sac. Fig. 2, division of two nuclei of embryo sac. Fig. 3, division of primary micropylar nucleus completed; primary chalazal nucleus still in resting stage. Fig. 4, 6-nucleate embryo sac. Fig. 5, mature embryo sac after polar fusion. Fig. 6, double fertilization. Fig. 7, first division of zygote; note persistence of both synergids in this case.

sperm nuclei into the sac. The other synergid persists for a while, and in rare cases both synergids seemed to remain intact for some time (fig. 7). Particular attention was paid to ascertain whether one of the synergid nuclei migrated downward to take part in triple fusion, as supposed by PACE, but no such evidence could be found. Figure 6 shows that double fertilization takes place normally.

Summary

1. The embryo sac of *Cypripedium spectabile* develops according to the *Allium*-type; and up to the 2-nucleate stage, my observations are in agreement with those of PACE.

2. The primary micropylar nucleus now divides twice to form the four micropylar nuclei, which typically organize into two synergids—the egg cell and the upper polar nucleus. The primary chalazal nucleus usually divides only once, but sometimes one or both of the resultant nuclei divide once again, resulting in 6-, 7-, or 8-nucleate embryo sacs of the kind seen in many other members of the Orchidaceae and certain of the Alismaceae (3). In all cases one nucleus from the chalazal group functions as the lower polar nucleus. The respective interpretations of PACE, RUTGERS, and the writer are shown in figure 8.

3. No indication could be found in favor of PACE's supposition that during fertilization one synergid nucleus moves downward and enters into triple fusion with the polar nucleus and the second male gamete.

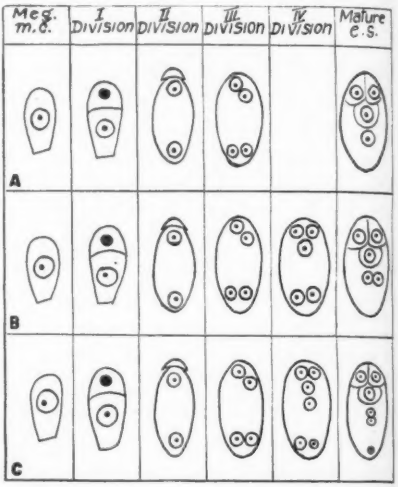


FIG. 8.—Diagrammatic representation of mode of development of embryo sac of *Cypripedium*, according to the interpretations of *A*, PACE (1907); *B*, RUTGERS (1923); and *C*, present writer.

Acknowledgment is due Dr. P. MAHESHWARI, Dacca University, for assistance and valuable suggestions.

18 NAGASANDRA ROAD
BASAVANGUDI
BANGALORE CITY
SOUTH INDIA

LITERATURE CITED

1. CARLSON, MARGERY C., Formation of the seed of *Cypripedium parviflorum*. BOT. GAZ. **102**: 295–301. 1940.
2. FRANCINI, E., Ricerche embriologiche e cariologiche sul genere *Cypripedium*. Nuovo Giorn. Bot. Ital. **38**:155–212. 1931.
3. JOHRI, B. M., Studies in the family Alismaceae. I. *Limnophyton obtusifolium* Miq. Jour. Ind. Bot. Soc. **14**:49–66. 1935.
4. MAHESHWARI, P., A critical review of the types of embryo sacs in angiosperms. New Phytol. **36**:359–417. 1937.
5. PACE, LULA, Fertilization in *Cypripedium*. BOT. GAZ. **44**:353–374. 1907.
6. PROSINA, M. N., Über die vom *Cypripedilum*-Typus abweichende Embryosackentwicklung von *Cypripedilum guttatum* Sw. Planta **12**:532–544. 1930.
7. PURI, V., A note on the embryo sac and embryo of *Moringa oleifera*. Proc. Ind. Acad. Sci. B. **1**: 279–282. 1935.
8. ———, Studies in the order Parietales. II. A contribution to the morphology of *Garcinia*

livingstonii T. Anders. Proc. Ind. Acad. Sci. B. 9:74–86. 1939.

9. RUTGERS, F. L., Embryo sac and embryo of *Moringa oleifera*. The female gametophyte of angiosperms. Ann. Jard. Bot. Buitenzorg 33:1–66. 1923.

10. SCHNARF, K., Embryologie der Angiospermen. Berlin. 1929.

11. ———, Vergleichende Embryologie der Angiospermen. Berlin. 1931.

12. ———, Contemporary understanding of the embryo sac development among angiosperms. Bot. Rev. 2:565–585. 1936.

13. TREUB, M., Le sac embryonnaire et l'embryon dans les angiospermes. Ann. Jard. Bot. Buitenzorg 24:1–27. 1911.

CURRENT LITERATURE

Physical Chemistry of Cells and Tissues. By RUDOLPH HÖBER; with the collaboration of DAVID I. HITCHCOCK, J. B. BATEMAN, DAVID R. GODDARD, and WALLACE O. FENN. Philadelphia: Blakiston Co., 1945. Pp. 676. Illustrated. $9.00.

The reappearance in completely new form of HÖBER's well-known work, *Physikalische Chemie der Zelle und der Gerwebe*, the last edition of which was published in 1926, is important to all who need to interpret the behavior of living cells or organisms in terms of physico-chemical principles.

This new book is written in eight sections, each the work of one of the five authors: Professor HÖBER himself, DAVID I. HITCHCOCK, J. B. BATEMAN, D. R. GODDARD, and W. O. FENN, who have combined to cover this extensive field of knowledge.

The first two sections treat concisely the physico-chemical principles which bear upon cellular phenomena. The emphasis here is upon the great strides made in the last decades in the elucidation of the submicroscopic structure of matter and the nature of the forces which bind particles together to form the units, large and small, the fibers, fibrils, surface films, and membranes which constitute the building units from which cellular architecture is derived. Diffusion, reaction velocities, the elements of thermodynamics, the properties of solutions, and the principles which underlie the phenomena of bioelectric potentials are all concisely reviewed.

Sections 3, 4, and 5, written by HÖBER himself, are concerned with the permeability properties of cells, and here the illustrative material is drawn in large part from the study of plant cells and includes a synopsis of the definitive works of COLLANDER and his school. The evidence which has made it necessary to visualize the surface of the protoplast as partaking simultaneously of permeability properties due to lipoid solubility and of properties due to passive diffusion through minute water-filled pores is reviewed.

Cell respiration is a field in which the student and the investigator must often ignore the somewhat arbitrary division between animal and plant material. The section devoted to a condensed review of this aspect of the subject is particularly interesting because it leads up to the final sections, which deal with mechanisms by which cells and tissues utilize their energy of metabolism to perform work.

The section dealing with contractile tissues in general, and muscle in particular, is naturally concerned almost wholly with animal systems; but the newer trends in this extensive subject are of interest to botanists, inasmuch as they indicate the possibility that the energy used by muscle in the performance of external work may be localized in specific reactions, and there is evidence that the energy liberated by oxidation may be stored up in phosphorylated compounds and transferred from compound to compound with the "high-energy phosphate" grouping.

HÖBER's concluding section, which deals with problems of "active transport" in plant and animal systems, is the climax of the book. After surveying the field, HÖBER authoritatively recognizes that it is a widespread function of living cells to "secrete" water or salts in a manner which demands that they must use energy to perform osmotic work. "Secretion" by the plant protoplast internally to the vacuoles is a classic case in point. This important problem in plants is treated as part of an even more general one, of which other examples are drawn from a wide range of biological systems. In all of them, however, the basic problem remains the same —how is the energy of metabolism used to perform osmotic work?

This book constitutes the most authoritative recent account of cells and tissues regarded as physico-chemical systems. Doubtless it will be freely consulted by cell physiologists for a long time to come. The subject matter, compactly contained in 635 pages, is liberally indexed and supplied with references to recent literature, either as footnotes or in lists of general references at the end of chapters, and the book itself is attractively produced.—F. C. STEWARD.

Fungicides and Their Action. By JAMES G. HORSFALL. Waltham, Mass.: Chronica Botanica Co.; New York: Steckert & Co., 1945. Pp. 239. Figs. 24. $5.00.

This second volume of the *Annales Cryptogamici et Phytopathologici* will be of special interest to those engaged in waging war against those fungi which injure and kill crop plants and to those interested in understanding the mechanisms and dynamics whereby fungicides bring about their injurious and killing effects.

The volume carries a foreword by DAVID FAIRCHILD, with an interesting reminiscence about the days when the mycological and fungicidal pattern of American phytopathology was set and when ALFRED FISCHER "denied that there are any such things as plant diseases caused by bacteria."

The author conceives of fungus control as warfare which is confronted with two basic problems—"(*a*) to procure the proper materials for killing the enemy, and (*b*) to deliver them to him in sufficient quantity when and where he is vulnerable." He focuses his task as a discussion of "these two problems in the light of chemistry and physiology of toxic action and of the mechanics of application." He succeeds well.

Chapter I is devoted to a historical introduction. Recorded use of fungicides goes back to the ancient Greeks, showing how mankind often hits upon the right track without understanding. The ancient Greeks knew nothing about the enemy they were combating with fungicides. Chapter II takes up definitions and basic concepts. These non-technological chapters are the least satisfactory part of the volume.

The remaining fourteen chapters present a satisfactory survey of the diffuse knowledge of one of the frontier fields of phytopathology. As the reviewer read these chapters he was again impressed by the wonderful opportunity which investigation of the injurious and killing effects of fungicides on fungi presents for study of the physics and chemistry of injury and death in plants. Viewed in this light, this volume should interest others than those professionally employed or interested in fungicidal control of fungus diseases in crop plants.

The volume closes with a comprehensive bibliography, a general index, and an authors' index. Its physical form is up to the excellent standard of the Chronica Botanica publications.—G. K. K. LINK.

An Introduction to the Taxonomy and Nomenclature of Fungi. By G. R. BISBY. Kew, Surrey: Imperial Mycological Institute, 1945. Pp. vii+117. $1.25.

As its title suggests, this little book is intended primarily for students who are beginning the study of systematic mycology. Following a brief introduction and discussion of principles, the remainder of the text is comprised under two parts—taxonomy and nomenclature. Under the first part a number of suggestions, many of them based directly on the personal experiences of the author, are presented on such topics as equipment, collecting, examining and recording, culturing, naming and describing, preserving, and publishing and illustrating.

In part 2 are given brief discussions of categories of fungi, synonomy, types and the type method, diagnoses, and rules. This part should prove helpful, not only to beginners but also to other botanists interested in nomenclature, since the International Rules of Botanical Nomenclature, not readily available in many libraries, are here presented in full.—J. M. BEAL.

Bibliography of References to the Literature on the Minor Elements, and Their Relation to Plant and Animal Nutrition. 6th Supplement to 3d ed. New York: Chilean Nitrate Educational Bureau, Inc., 1945. Pp. 103.

In this supplement are found more than 700 abstracts and references. These are concerned with 52 chemical elements and 95 plants and plant groups. The four indexes of the fifth edition—author, element, animal nutrition, and botanical—are continued. The yearly issuance of the supplement is helpful to those wishing to keep abreast of the literature of mineral nutrition.—S. V. EATON.